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RE-EVALUATION OF PROTEIN REQUIREMENTS OF GROWING-FATTENING SWINE AS INFLUENCED BY FEEDING AN ANTIBIOTIC[1, 2]

D. V. Catron, A. H. Jensen, P. G. Homeyer, H. M. Maddock and G. C. Ashton[3]

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GENERAL observations of farm practice have shown that many hog producers have obtained satisfactory growth in swine when feeding rations containing lower levels of protein than those commonly recommended. The research leading to these recommendations was conducted almost a decade ago. In light of recent advances in animal nutrition it seemed advisable to re-evaluate the protein requirements of swine.

Keith and Miller (1939, 1941) fed rations varying in protein content from 12 to 27 percent and found that maximum and most economical gains from weaning to 75 pounds were made by those pigs fed the highest percent of protein. Twenty-two percent was recommended from weaning to 70 pounds, 17 to 20 percent from 75 to 125 pounds and 15 percent from 125 to 200 pounds. A series of studies by Carroll and Burroughs (1939) substantiated these conclusions. Mitchell (1939), using the same rations as Carroll and Burroughs, but employing the N-retention technique of measuring efficiency, stated that 40- to 50-pound pigs required more than 26 percent protein, 100-pound pigs in excess of 17 percent, 150-pound pigs 17 percent and 150- to 200-pound pigs 15 percent protein for maximum growth.

However, Crampton and Ashton (1942) proposed 15 to 18 percent protein for pigs from 30 to 100 pounds and 14 to 16 percent for 100 to 200 pounds. Then Ferrin (1946) demonstrated that a 12 percent protein ration was not sufficient to permit satisfactory growth in weanling pigs.

Satisfactory explanations for discrepancies in the suggested protein requirement of the pig are not readily apparent. Nutritional values of the known vitamins, especially the recently identified vitamin B_{12}, and

[1] Journal Paper No. J-2053 of the Iowa Agricultural Experiment Station, Animal Husbandry Department, Ames, Iowa. Project 930.

[2] The vitamins and antibiotics were supplied by Merck and Co., Inc., Rahway, New Jersey; Lederle Laboratories, Pearl River, New York and Standard Brands, Inc., New York, New York.

[3] Acknowledgment is made to Don Quinn, Animal Nutrition farm foreman and his associates for their assistance.

their possible interrelationships with protein needs must be considered. Increasing protein intake by supplying greater quantities of animal protein ingredients or oil meals in rations could increase non-protein dietary factors, such as water-soluble vitamins, which may be responsible for the increased rate of gain after the protein level has been raised beyond that point required for meeting the amino acid requirements of the pig.

In addition, the current practice of adding antibiotics to swine rations further complicates the problem. Lepley *et al.* (1950), Catron (1951) and several other investigators have demonstrated increased growth rate with these factors. Cunha (1950) suggested that the protein requirements of swine might be reduced by feeding antibiotics.

The investigation reported herein was conducted to re-evaluate the protein requirements of growing-fattening pigs in concrete drylot with and without the antibiotic, aureomycin.

Experimental Procedure

The initial protein levels selected for this study were 20, 18, 16 and 14 percent. When the pigs reached an average weight of 75 pounds the protein content of the rations was reduced 3 percentage points to 17, 15, 13 and 11 percent, and at 150 pounds the levels were further reduced to 14, 12, 10 and 8 percent protein. To determine the effect of an antibiotic on the protein requirement of the pig, two of the four lots of pigs on each set of protein levels received ten mg. of aureomycin hydrochloride per pound of ration.

Because of the limitations imposed by litter size, a split-plot design was used so that the effect of different protein levels and the interaction of aureomycin with protein level could be measured as precisely as possible. Each of the two replicates used consisted of two groups of four lots with a random determination as to which group received aureomycin. Within each group of four lots, the four levels of protein were assigned at random. For each group of four lots, eight outcome groups of four pigs were selected. In a few cases where outcome groups could not be made up of littermates, other pigs were selected for uniformity with respect to breeding, previous treatment, weight, condition, and age. One pig from each of the outcome groups was randomly allotted to each lot. The 128 Duroc pigs used had been in continuous drylot with no previous antibiotic treatment. The four groups of four lots were started on experiment within a period of ten days. Average

starting lot weights ranged from 29.9 to 37.5 pounds per pig, and the average age ranged from 57 to 59 days. The pigs were wormed with sodium fluoride and sprayed with benzene hexachloride at the beginning of the experiment. Feed and water were available *ad libitum* and the pigs were confined to concrete pens during the entire feeding period. The pigs were weighed individually at two-week intervals and as often as necessary to permit changing protein levels. Each lot of pigs was removed from test at an average weight of approximately 200 pounds per pig.

The rations were composed of ground yellow corn, solvent soybean oil meal, special steamed bonemeal, iodized calcium carbonate, a trace mineral mixture, iodized salt and a vitamin premix contributing the following per pound of ration: 5 mg. calcium pantothenate, 250 mg. choline chloride, 2.5 mg. folic acid, 10 mg. niacin, 1.5 mg. pyridoxine. 1 mg. riboflavin, 1.5 mg. thiamin hydrochloride, 10 mcg. vitamin B_{12}, 300 I.U. vitamin A and 400 I.U. vitamin D_2. The trace mineral mixture contributed to the ration in ppm: iron 70, copper 4.8, cobalt 1.6, manganese 59 and zinc 4.4. The soybean oil meal was a blend of equal parts of three different manufacturers' solvent processed meals. The various protein levels were obtained by adjusting the corn and soybean oil meal content of the ration based on chemical analysis. The calculated calcium and phosphorus content of all the rations were held constant by adjusting the amount of steamed bonemeal and calcium carbonate. Table 1 shows the protein content of the rations and the average percent protein consumed by the pigs fed the various rations.

Six barrows from each of the four protein-level treatments, including both with and without aureomycin, were slaughtered. Back fat, body length, body depth and total percent of lean in selected cuts were measured.

The several groups of data in this test were analyzed statistically according to the scheme shown in table 2. All values reported as significant are at the 5 percent probability level.

Results and Discussion

A discussion of the data obtained from this experiment naturally divides itself into four weight periods of the pigs: (1) weaning to 75 pounds, (2) 75 to 150 pounds, (3) 150 to 200 pounds, and, (4) weaning to 200 pounds.

TABLE 1. PROTEIN CONTENT OF RATIONS IN PERCENT

	Protein Levels (%) [1]			
Initial to 75 lb.	20	18	16	14
75 to 150 lb.	17	15	13	11
150 to 200 lb.	14	12	10	8
	(As found from ration samples) [2]			
No Aureomycin				
	20.2	18.9	16.3	14.2
	17.6	15.0	13.6	11.3
	15.3	12.3	10.3	8.1
10 mg. Aureomycin per lb. of ration				
	20.3	18.3	16.2	14.3
	17.2	15.6	13.0	11.2
	16.0	12.4	10.7	8.9
	(Average percent protein consumed) [3]			
No Aureomycin				
	16.6	14.7	12.7	10.8
10 mg. Aureomycin per lb. of ration				
	16.6	14.7	12.7	10.8

[1] The rations as calculated from ingredient analyses contained from 0.1 to 0.3 percentage points more protein.
[2] Analyzed in accordance with official A.O.A.C. methods (1945).
[3] Calculated from the amount of each ration consumed by the 16 pigs on each set of protein levels with and without antibiotic over the entire feeding period.

TABLE 2. ANALYSIS OF VARIANCE OF LOT-MEAN DAILY GAINS FROM WEANING TO 200 POUNDS

Sources of Variation	Degrees of Freedom	Mean Square	F Value
Replicates	1	—	
Antibiotic	1	0.05448	
Error (a)	1	0.00324	
Protein Levels			
No Antibiotic	3	0.00703	
Linear Component [1]	1	0.00009	0.08
Quadratic Component	1	0.01324	12.19 *
Cubic Component	1	0.00578	4.62
Antibiotic	3	0.00013	
Linear Component	1	0.00011	0.09
Quadratic Component	1	0.00002	0.01
Cubic Component	1	0.00026	0.21
Error (b)	6	0.00125	
Total	15	—	

* Significant at P=0.05.
[1] For basis and interpretation of regressions see Snedecor (1946), Cochran and Cox (1950) and Kempthorne (1952).

Data on daily gain, daily feed, and feed per 100 pounds gain are presented in tables 3, 4 and 5, respectively, as averages for each of the eight treatments. In figure 1, the average daily gains of the pigs by treatments from weaning to 200 pounds are shown graphically.

TABLE 3. SUMMARY OF AVERAGE DAILY GAINS BY WEIGHT PERIODS [1]

		Protein Levels (%)				
Initial to 75 lb.		20	18	16	14	
75 to 150 lb.		17	15	13	11	Av.
150 to 200 lb.		14	12	10	8	
		(Pounds)				
Initial to 75 lb.						
No Aureomycin		1.21	1.29	1.26	1.23	1.25
10 mg. Aureomycin		1.31	1.28 [2]	1.34	1.26	1.30
Average		1.26	1.28	1.30	1.24	
75 to 150 lb.						
No Aureomycin		1.53	1.58	1.67	1.64	1.60
10 mg. Aureomycin		1.79	1.73 [2]	1.83	1.84	1.80
Average		1.66	1.65	1.75	1.74	
150 to 200 lb.						
No Aureomycin		1.81	1.81	2.02	1.54 [3]	1.80
10 mg. Aureomycin		1.94	2.03 [2]	1.80	1.88	1.91
Average		1.87	1.92	1.91	1.71	
Initial to 200 lb.						
No Aureomycin	Rep. I	1.50	1.59	1.64	1.52	1.55
	Rep. II	1.53	1.53	1.62	1.48	
Aureomycin	Rep. I	1.64	1.64	1.70	1.62	1.67
	Rep. II	1.71	1.68	1.65	1.70	
Average		1.60	1.61	1.65	1.58	

[1] Each figure (excluding averages) represents an average of two lots of eight pigs each or a total of 16 pigs.

[2] The gain of one pig which died of a physiological disorder not attributable to nutrition was estimated (Snedecor 1946).

[3] The gain of one pig was estimated from 150 to 200 lb. (Snedecor 1946) as it did not respond normally.

The use of the split-plot design, for the reasons previously stated, severely limits the precision in measuring the effects of the whole plot treatments, antibiotic versus no antibiotic. In this case with only one degree of freedom for error, no attempt will be made to compare statistically the average effect of aureomycin versus no aureomycin. The discussion will be confined, therefore, to the average effects of protein levels and their interactions with the antibiotic.

Initial to 75 Pounds Weight

No significant differences in daily gains resulted by varying the protein levels either in the presence or absence of the antibiotic during this period.

The variations in daily feed intake for all treatments considered above were likewise found to be no greater than chance variations.

TABLE 4. SUMMARY OF AVERAGE DAILY FEED INTAKE BY WEIGHT PERIODS [1]

	Protein Levels (%)				
Initial to 75 lb.	20	18	16	14	
75 to 150 lb.	17	15	13	11	Av.
150 to 200 lb.	14	12	10	8	
			(Pounds)		
Initial to 75 lb.					
No Aureomycin	3.44	3.32	3.54	3.23	3.38
10 mg. Aureomycin	3.10	3.04	3.50	3.25	3.22
Average	3.27	3.18	3.52	3.24	
75 to 150 lb.					
No Aureomycin	6.18	6.34	6.34	6.14	6.25
10 mg. Aureomycin	6.50	6.32	6.53	6.66	6.50
Average	6.34	6.33	6.43	6.40	
150 to 200 lb.					
No Aureomycin	8.13	8.27	8.65	7.13	8.04
10 mg. Aureomycin	8.44	8.69	7.84	8.27	8.31
Average	8.28	8.48	8.24	7.70	
Initial to 200 lb.					
No Aureomycin	5.89	5.97	6.06	5.56	5.87
10 mg. Aureomycin	5.93	5.83	5.94	5.96	5.91
Average	5.91	5.90	6.00	5.76	

[1] Each figure (excluding averages) represents an average of two lots of eight pigs each or a total of 16 pigs.

The data for feed per 100 pounds gain show considerable apparent variability, especially in the absence of the antibiotic. The values for the different protein levels in the presence of the antibiotic were not significantly different but those in the no-antibiotic group were. Thus the feed efficiency was different on the two treatments but its particular behaviour in the absence of the antibiotic is not readily explainable.

There is no evidence in these data to indicate that a protein level higher than the 14 percent is superior in any respect for the growth of weanling pigs fed a corn-soybean oil meal ration under the conditions of this experiment.

TABLE 5. SUMMARY OF FEED REQUIRED PER 100 POUND GAIN BY WEIGHT PERIODS [1]

	Protein Levels (%)				
Initial to 75 lb.	20	18	16	14	
75 to 150 lb.	17	15	13	11	Av.
150 to 200 lb.	14	12	10	8	
			(Pounds)		
Initial to 75 lb.					
No Aureomycin	285	258	280	261	271
10 mg. Aureomycin	238	244	260	257	250
Average	261	251	270	259	
75 to 150 lb.					
No Aureomycin	404	401	380	374	390
10 mg. Aureomycin	364	376	357	363	365
Average	384	388	368	368	
150 to 200 lb.					
No Aureomycin	448	457	428	482	454
10 mg. Aureomycin	435	435	435	441	436
Average	441	446	431	461	
Initial to 200 lb.					
No Aureomycin	390	383	372	374	380
10 mg. Aureomycin	354	360	355	359	357
Average	372	371	363	366	

[1] Each figure (excluding averages) represents an average of two lots of eight pigs each or a total of 16 pigs.

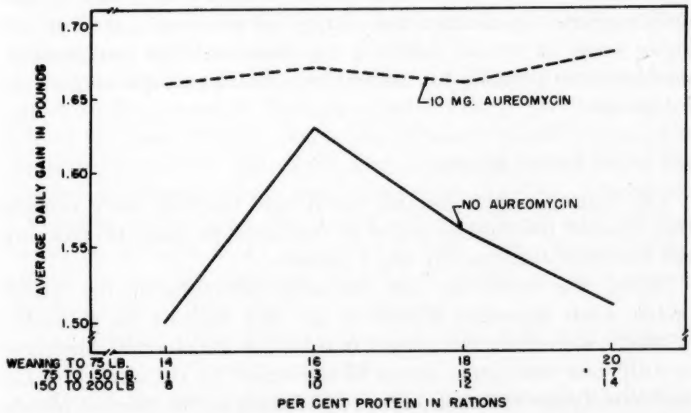

Figure 1. Mean daily gains of pigs from weaning to 200 pounds on different levels of protein with and without an antibiotic.

75 to 150 Pounds Weight

The average weights of the lots of pigs had a maximum range of only 5 pounds when the protein levels of the rations were changed. Thus the average initial weights for this feeding period were relatively uniform.

The average daily gains made by the pigs on the different protein levels in this weight period tend in the opposite direction to what many previous reports would lead one to expect, namely, that the higher percent protein groups would gain more rapidly. While the apparent trend is in favor of the lower protein groups, this trend was found not to be significant. The results indicate that a protein level of 11 percent is adequate for pigs over 75 pounds in weight when fed a corn-soybean oil meal ration either in the presence or absence of the antibiotic at the level fed.

In the case of daily feed intake during this period, a situation similar to that of rate of gain prevailed.

As in the first period, however, there were significant differences in feed per 100 pounds gain during this period. In the case where no antibiotic was fed, there was a steady increase in feed efficiency as the percent protein was lowered. This did not occur in the antibiotic-fed group. There the efficiency was relatively constant for the lots of pigs fed the different protein levels. Continuation of excessively loose feces in the pigs fed higher levels of protein without auromycin probably caused the accompanying decrease in feed efficiency. This view is partially supported by the fact that no pigs fed aureomycin, even on the higher levels of protein, exhibited this looseness. This, undoubtedly, would account partially for greater feed efficiency in the aureomycin fed groups.

150 to 200 Pounds Weight

The starting weights for this period were but little more variable than those of the previous period as the maximum range between any two treatment averages here was 6 pounds.

During this period the pigs responded differently on the several protein levels depending whether or not they received the antibiotic. When the aureomycin was present in the ration the observed differences in daily gain were found not to be significant. In the absence of the antibiotic, the growth rates differed significantly on the different protein levels.

The daily gain of 1.88 pounds made by the antibiotic 8 percent

protein groups compared to 1.54 pounds for the no-antibiotic groups suggests that under conditions of low protein intake the antibiotic exerted a protein "sparing-like" effect.

Feed intake is more variable here than in any period so far considered. The presence of the antibiotic did not, on the average, increase daily feed intake significantly. In its absence, however, feed intakes varied significantly over the range of protein levels. The 8 percent protein group consumed over a pound less feed per day than the other three protein-level groups.

None of the differences in feed per 100 pounds gain were found to be significant.

Initial to 200 Pounds Weight

Growth response over the entire feeding period showed significant differences among the different protein levels fed depending upon whether or not the antibiotic was present in the ration. The daily gains varied in a parabolic manner in the non-antibiotic groups with the peak of the curve at or near the 16 percent protein level as is shown in figure 1. When the antibiotic was fed no significant differences in daily gains were observed on the different protein levels. The daily gains as presented in figure 1 strongly suggest that the feeding of the antibiotic has exerted a "sparing-like" effect on protein. Yet, the average percent protein consumed by the pigs fed with or without antibiotic were the same (table 1) based on the actual amounts of each ration consumed by the 16 pigs on each set of protein levels with and without antibiotic from weaning to 200 pounds.

The consumption of a ration averaging 12.7 percent protein throughout the feeding period by those pigs starting with 16 percent protein is in close agreement with the results of a previous 12 week trial in which the pigs were self-fed, free-choice, shelled corn, whole oats and a well fortified supplement which was highly mineralized to decrease the palatability. These latter pigs gained 1.50 pounds per day and consumed on the average 11.15 percent protein. Their weekly average protein consumption decreased from 12.88 percent to 10.34 percent during the period from weaning to 150 pounds.

No significant differences were found in either daily feed intake or feed efficiency due to ration treatment.

Although the pigs fed aureomycin required 23 pounds less feed to make 100 pounds of gain when compared to those not receiving antibiotic, this difference was not statistically significant.

Data presented in table 6 are contrary to U.S.D.A. reports (1946), which showed that a greater proportion of lean tissue to fat tissue was present when rations averaging approximately 18 percent protein were fed than when 13 percent protein rations were fed. No significant differences existed in back fat measurements between the pigs fed the different levels of protein. Dressing percentage, body length, body depth and percent of lean were not significantly influenced by the protein content of the ration or by the absence or presence of antibiotic. All of these animals, however, were very fat. The average percent of

TABLE 6. SUMMARY OF CARCASS MEASUREMENTS BY PROTEIN LEVELS AND AUREOMYCIN TREATMENT

	No. Pigs	Av. Live Wt.	Av. Dressing Percent	Av.[1] Length Carcass (Inches)	Av.[2] Depth Carcass (Inches)	Av.[3] Back Fat Depth (Inches)	Av. Percent Lean
Protein Levels (%)							
20-17-14	6	200	80.1	28.6	13.7	1.80	26.7
18-15-12	6	197	80.5	28.5	13.1	1.80	24.3
16-13-10	6	199	81.6	28.5	13.5	1.85	28.8
14-11- 8	6	200	80.5	28.3	13.3	1.85	28.0
No Aureomycin	13	201	80.0	28.5	13.4	1.84	27.4[4]
10 mg Aureomycin	11	197	81.5	28.5	13.4	1.83	26.7

[1] Length measured from the first rib to the point of the aitch-bone.
[2] Depth measured at the seventh rib.
[3] Measurements taken at the first, seventh, last rib and last lumbar.
[4] Eleven pigs in this average.

lean in the cross-section of the 24 carcasses at the last rib was only 26.95. This poses the question, would a leaner type hog produce a leaner carcass on the higher protein levels?

Fecal samples were collected from representative pigs in groups which received 14 percent protein, with and without antibiotic and 20 percent protein, with and without antibiotic. There was no apparent correlation in the number of intestinal bacteria and the rate of gain of pigs in this experiment. With the 20 percent level of protein, the addition of antibiotic noticeably increased the total microflora count. When antibiotic was absent, the increase in protein composition of the ration appeared to have little effect on the numbers of enteric microorganisms.

Research recently completed with Landrace X Duroc pigs confirms

these findings that lower protein levels than presently recommended produce satisfactory performance when rations are properly balanced.

Summary and Conclusions

A total of 128 Duroc pigs were fed a corn-soybean oil meal ration supplemented with minerals and vitamins, including vitamin B_{12}, from weaning to 200 pounds in drylot.

A 2 x 4 factorial experiment in a split-plot design with 0 and 10 mg. of aureomycin hydrochloride as the whole plots and the initial protein levels of 20, 18, 16 and 14 percent as the sub-plots was conducted to determine whether previously recommended protein levels are too liberal for present feeding practice and to study the effects of antibiotic supplementation on the protein needs of the pig. Each protein level was reduced 3 percentage units when the pigs reached 75 pounds and again at 150 pounds.

In the absence of antibiotic, the rate of gain for the entire feeding period varied significantly ($P=0.05$) among the several sets of protein levels. The results suggest that in the absence of an antibiotic the 16–13–10 percent protein level combination supplied the pig's needs for protein from weaning to market, whereas in the presence of the antibiotic the 14–11–8 percent level combination produced gains equivalent to higher levels of protein. Contrary to previous recommendations, higher levels of protein are in excess of the pigs' needs if rations are balanced in respect to non-protein dietary factors.

Aureomycin, added at the rate of 10 mg. per pound of ration, appeared to exert a protein "sparing-like" effect on the lower protein levels.

Pigs receiving antibiotics in their ration gained an average of 0.12 pound more per day and consumed 23 pounds less feed per 100 pounds of gain than those pigs not receiving the antibiotic.

No significant differences existed among the levels of protein fed or between antibiotic and no-antibiotic treatments in respect to back fat depth, length or depth of body or percent of lean when measured on 24 representative carcasses.

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PROTEIN AND VITAMIN SUPPLEMENTS FOR GROWING-FATTENING PIGS ON LADINO CLOVER AND ON ALFALFA[1]

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WORK at the Kentucky Agricultural Experiment Station and other stations has established the value of dried corn distillers' solubles as an important component of rations for growing-fattening pigs fed in drylot.

Fairbanks *et al.* (1944, 1945) and Krider *et al.* (1944, 1950) have shown that the nutritive qualities of a basal ration composed of ground yellow corn, wheat flour middlings, soybean meal, fishmeal tankage, minerals and fortified cod-liver oil was improved by the addition of 6 percent dried corn distillers' solubles when fed to growing-fattening pigs in drylot. Robison (1949) found that dried distillers' grain solubles, when fed at the rate of 20 percent of the protein supplement or 6 percent of the total ration to growing-fattening pigs in drylot, improved a ration of yellow corn, soybean oilmeal, ground alfalfa, minerals and irradiated yeast. Wilford (1948) demonstrated that a protein supplement mixture of 4 parts of soybean oilmeal and 1 part distillers' solubles was comparable to the "Wisconsin Trio" mixture of 2 parts of tankage, 1 part of soybean oilmeal and 1 part of ground alfalfa meal for growing-fattening pigs in drylot.

Catron and Culbertson (1949) reported the value of APF concentrate when added to a ration of yellow corn, soybean oilmeal, dehydrated alfalfa meal, minerals and vitamins A and D and fed to growing-fattening pigs in drylot. Catron and Wallace (1949) reported pigs grazing alfalfa-brome and alfalfa and clover pastures made faster daily gains when an APF concentrate was added to their ration. Vestal (1950) reported that the addition of an APF supplement to soybean oilmeal fed free-choice with corn to growing-fattening pigs grazing alfalfa pasture and oats and rape pasture resulted in increased gains.

The object of this experiment was to compare meat scraps, dried corn distillers' solubles and a vitamin B_{12} aureomycin feed supplement when fed with expeller soybean oilmeal and yellow corn to growing-fattening pigs grazing alfalfa pasture and ladino clover pasture.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

Experimental Procedure

Trial I (Summer, 1950)

Six lots of seven purebred Duroc and Chester White pigs were group fed from an average initial weight of 59 pounds to an average final weight of 200 pounds. Pigs were evenly allotted according to breed, sex, weight and litter. The average of three day weights was taken for initial and final weight. Pigs were weighed periodically at 14 day intervals. The rations given in table 1 were self fed. A simple mineral mixture of 2 parts limestone, 2 parts of steamed bone meal and 1 part

TABLE 1. COMPOSITION OF RATIONS FED IN TRIALS I AND II

Ingredients	Ration Number		
	1	2	3
		(parts)	
Ground yellow corn	76.5	71.5	74.5
Expeller soybean oilmeal	17.5	22.5	20.5
Meat Scraps	6.0	....	....
Dried corn distillers' solubles	....	6.0	....
APF premix [1]	....	....	5.0
Total	100.0	100.0	100.0
Percent protein [2] (Calculated)	17.97	18.06	19.04

[1] Five pounds of Lederle's APF Feeding Supplement No. 5 was premixed with 95 pounds of soybean oilmeal in trial I. In trial II, 10 pounds of Lederle's Aurofac was premixed with 90 pounds of soybean oilmeal.

[2] Crude protein was adjusted downward as pigs increased in weight: 18 percent until pigs weighed 100 pounds, 15 percent from 100 pounds to 125 pounds, and 13 percent from 125 pounds to 200 pounds.

of salt was self-fed. Loose salt was also available free-choice. Water was supplied each lot by an automatic fountain.

Three lots of pigs grazed ladino clover and the other three lots grazed alfalfa. No attempt was made to measure the quantity of pasture consumed. Each pasture plot was four-tenths of an acre in size and provided an abundant supply of pasture for the pigs during the entire feeding period.

Trial II (Summer, 1951)

The design of the second trial was identical to trial I except that the vitamin B_{12} aureomycin supplement was fed at 0.5 percent of the total ration as compared to 0.25 percent of the total ration in trial I.

Seven purebred Hampshire or Duroc pigs averaging approximately

36 pounds were assigned to the three lots of alfalfa and three lots of ladino clover and were fed to an average final weight of 200 pounds.

These pigs, like those used in trial I, were produced by sows that were fed commonly accepted adequate gestation and lactation rations. All pigs were moved to a clean bluegrass pasture 10 days following farrowing and remained there until being placed on this experiment.

Results and Discussion

Results of the two trials are presented in tables 2 and 3. When meat scraps or dried corn distillers' solubles were replaced by APF in ration

TABLE 2. RESULTS OF TRIAL I

Pasture	Ladino Clover			Alfalfa		
Lot Number	1	2	3	4	5	6
Ration Number Fed	1	2	3	1	2	3
Number of pigs	7	7	7	7	7	7
Av. initial wt. (lb.)	60.3	58.6	58.7	58.1	60.3	59.8
Av. final wt. (lb.)	198.4	194.7	198.3	196.1	201.4	203.7
Days on feed	74	74	74	74	74	74
Av. daily gain (lb.)	1.87	1.84	1.89	1.86	1.91	1.94
Av. daily feed (lb.)	6.19	5.99	6.04	6.00	6.10	6.05
Feed consumed per 100 lb. gain (lb)	331.75	325.49	320.37	321.53	320.04	311.52

number 3, slightly more rapid gains were noted on both pastures and also more efficient gains except in the case of lot 6, trial II. Dried corn distillers' solubles proved to have a slight advantage over meat scraps in efficiency of gains when fed to pigs grazing both pastures. In trial II the difference in average daily gain between ration 3 and rations 1 or 2 was greater than in trial I, suggesting that by doubling the level of the vitamin B_{12} aureomycin supplement in the ration, growth response was increased. In trial I the three lots of pigs grazing alfalfa pasture made slightly more efficient gains than the corresponding three lots of pigs grazing ladino clover. However, in trial II, when rations 1 and 3 were fed to pigs grazing ladino clover, more efficient gains were produced than when the same rations were fed to pigs grazing alfalfa. Pigs fed ration 2 and grazing alfalfa consumed slightly less feed per unit of gain than those pigs receiving ration 2 and grazing ladino clover.

There were no significant differences in gains between the different treatments (Snedecor, 1946).

Summary

Six percent of meat scraps, 6 percent of dried corn distillers' solubles, and 0.25 percent or 0.5 percent vitamin B_{12} aureomycin supplement proved to be comparable to one another when fed with ground yellow corn, expeller soybean oilmeal, limestone, steamed bone meal and salt when fed growing-fattening pigs grazing either ladino clover pasture or alfalfa pasture. No significant differences in gains were noted between six lots of pigs in either of two trials.

TABLE 3. RESULTS OF TRIAL 2

Pasture	Ladino Clover			Alfalfa		
Lot Number	1	2	3	4	5	6
Ration Number Fed	1	2	3	1	2	3
Number of pigs	7	7	7	7[1]	7	7[2]
Av. initial wt. (lb.)	38	35	35	38	36	37
Av. final wt. (lb.)	208	203	205	199	207	207
Days on feed	112	112	106	106	112	106
Av. daily gain (lb.)	1.52	1.50	1.60	1.46	1.53	1.65
Av. daily feed (lb.)	5.17	5.09	5.28	5.15	5.16	5.83
Feed consumed per 100 lb. gain (lb.)	340.7	339.6	330.4	347.4	337.8	352.9

[1] One pig removed 6/19/51, pneumonia.
One pig died 6/29/51, hog cholera.
[2] One pig died 7/31/51, heat stroke.

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THE RELATIONSHIP OF PROTEIN TO PANTOTHENIC ACID AND VITAMIN B_{12} IN THE GROWING PIG[1]

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PREVIOUS work by Luecke, McMillen and Thorp (1950) indicated that pantothenic acid deficiency could be produced in growing pigs fed a corn-soybean meal type ration containing 4.21 mg. of pantothenic acid per pound and 16.4 percent protein. The results of this work were obtained before vitamin B_{12} became generally available. Unpublished results from this experiment station obtained in the summer of 1950 indicated that severe pantothenic acid deficiency could not be produced in pigs fed a corn-soybean meal type ration containing 12.5 μg. of vitamin B_{12} per pound, 18.3 percent protein and 4.15 mg. of pantothenic acid per pound of ration. These results indicated a possible sparing effect of vitamin B_{12} on the pantothenic acid requirement of the pig.

Recently, Yacowitz, Norris and Heuser (1951) obtained definite evidence that vitamin B_{12} exerted a sparing effect on the pantothenic acid requirement of chicks. Furthermore, these same workers found that pantothenic acid also showed a sparing effect on the vitamin B_{12} requirement for growth of normal chicks.

Level of protein may also be an important factor in pantothenic acid deficiency as evidenced by the work of Nelson and Evans (1945) and Nelson, Van Nouhuys and Evans (1947) with the rat. In this instance it was found that high protein diets exerted a sparing effect on the pantothenic acid requirement of this species.

Experimental

The pigs used in this experiment were crossbreds (Duroc x Poland x Hampshire). They were creep-fed a 16 percent protein feed mixture on good legume pasture and were approximately six weeks of age at the

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start of the trial. For the duration of the experiment the animals were kept on concrete floors, bedded with wood shavings and were self fed.

The composition of the two rations used in the experiment is shown in table 1. The fish solubles used in the trial was assayed microbiologically for vitamin B_{12} according to the method of Skeggs *et al.* (1948). Thiomalic acid was used in the medium as a reducing agent. It was found that the addition of the fish solubles provided only 0.74 μg. of vitamin B_{12} per pound of ration.

Since the low protein basal ration A contained significantly less pantothenic acid than ration B, calcium pantothenate was added in

TABLE 1. COMPOSITION OF BASAL RATIONS [1]

Ingredients	Ration	
	Basal A [2]	Basal B
	Percent	Percent
Corn	80	73
Soybean oil meal (solvent)	15	22
Fish solubles (menhaden)	2	2
Limestone	1.4	1.4
Dicalcium phosphate (Pura-Phos)	1.0	1.0
Iodized salt	0.5	0.5
Trace mineral premix (Cal. Carb. Corp.)	0.1	0.1
Crude protein	14.0	17.8

[1] Both rations received supplementary niacin and riboflavin in the amounts of 6 mg. and 2 mg. per pound of feed respectively. Ample amounts of vitamins A and D were also added to both rations.

[2] Calcium pantothenate was added to ration A in the amount of 0.5 mg. per pound of feed in order to equalize the pantothenic acid content of both rations.

the amount of 0.5 mg. per pound of ration. Thus, the pantothenic acid content of rations A and B was found to be 4.11 and 4.07 mg. per pound respectively. The pantothenic acid assays were carried out microbiologically using the method of Skeggs and Wright (1944). Mylase P was used to liberate pantothenic acid from the feeds.

Recently, newer methods for the liberation of pantothenic acid from various natural materials have been used. These methods involved the use of two enzymes, intestinal phosphatase and a pigeon liver enzyme. Using the method of Novelli and Schmetz (1951) for total pantothenic acid it was found that basal rations A and B contained 4.97 and 4.98 mg. of pantothenic acid per pound.

Results and Discussion

The results of the trial together with the various experimental treatments are shown in table 2. It can be seen that the pigs in lot 1, fed the low protein basal ration A, grew very poorly. The supplementation of basal ration A with vitamin B_{12} (lot 2) did not result in any marked increase in daily gain over that of lot 1. However, the addition of

TABLE 2. RESULTS OF PANTOTHENIC ACID, B_{12} AND PROTEIN TRIALS

(Fifteen week experimental period)

Lot No.	Ration and Supplement	No. pigs [1]	Av. initial wt.	Av. daily gain	Av. daily feed	Feed per lb. gain
			lbs.	lbs.	lbs.	lbs.
1	Basal A	9	21.0	0.67	2.36	3.54
2	Basal A + B_{12} [2]	9	20.9	0.75	2.62	3.48
3	Basal A + Cal. pan.[3]	8	20.9	1.09 *	3.42	3.14
4	Basal A + B_{12} + Cal. pan.	8	21.0	1.10 *	3.43	3.12
5	Basal B	9	20.9	1.12	3.64	3.24
6	Basal B + B_{12}	8	20.9	1.13	3.63	3.20
7	Basal B + Cal. pan.	9	20.8	1.22	3.75	3.08
8	Basal B + B_{12} + Cal. pan.	9	21.0	1.32	4.37	3.32

* Daily gains significant over lot 1 (P=0.05).

[1] One pig each from lots 3, 4 and 6 were not growing to any appreciable extent and were removed from the trial, necropsied and found to have had severe pneumonia.

[2] Vitamin B_{12} was added as the Merck concentrate SP 622 in amounts supplying 25 μg. per pound of feed.

[3] Calcium pantothenate was added to the designated rations at levels of 4 mg. per pound of feed.

calcium pantothenate to basal ration A (lot 3) resulted in a very marked increase in daily gain as well as a marked increase in feed efficiency. From the results shown in table 2 it is apparent that the growth response of the pigs in lot 4, fed basal ration A supplemented with both vitamin B_{12} and calcium pantothenate, was due entirely to the effect of the latter vitamin.

The growth response of the pigs in lot 5 (table 2) fed the higher protein basal ration B was considerably greater than that of the animals in lot 1 fed the low protein basal ration A. The addition of vitamin B_{12} to basal ration B (lot 6) did not result in any significant increase in average daily gain when compared to the basal group in lot 5. The addition of calcium pantothenate to basal ration B (table 2) resulted in a slight, though not statistically significant, increase in average daily

gain. The increase was not nearly so marked as that noted in comparing lots 1 and 2. When vitamin B_{12} and calcium pantothenate were added to basal ration B (lot 8), there was no statistically significant increase in average daily gain when compared with lot 7.

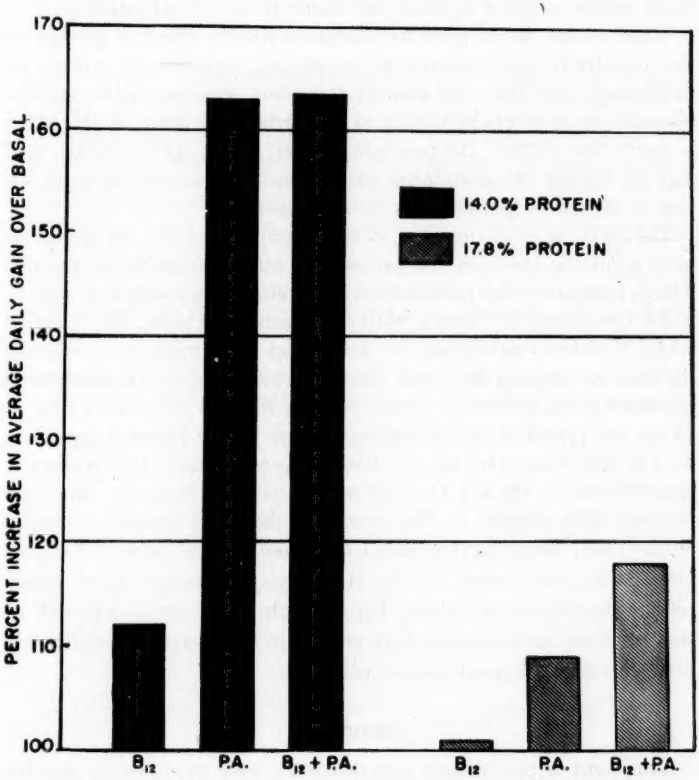

Figure 1. Comparative growth response obtained by adding calcium pantothenate and vitamin B_{12} single and combined to basal rations A and B.

Figure 1, a bar graph, shows the comparative growth responses obtained by adding calcium pantothenate and vitamin B_{12} singly or combined to both basal rations A and B. The data in the graph were obtained by considering the average daily gain of the pigs in lot 1 fed basal ration A to be 100 percent, and then calculating the average daily gains of lots 2, 3 and 4 as percentage increases in average daily gain

over that of the basal. The same thing was done for the lots fed basal ration B (lot 5) and the supplements (lots 6, 7 and 8).

Figure 1 clearly shows the very marked response to calcium pantothenate in the pigs fed the low-protein ration A as compared with the much smaller response noted in the higher protein basal ration.

These results would seem to indicate a sparing effect of protein on the pantothenic acid requirement of the pig. Of course, it can not be determined from this trial whether this effect was due to the soybean oil meal *per se* or to the protein of the soybean oil meal. If this effect is due to the protein, the possibility exists, that a specific amino acid may be sparing the pantothenic acid requirement of the pig as in the case of the niacin sparing effect of tryptophan.

The addition of vitamin B_{12} to both basal rations did not appear to have a sparing effect on the pantothenic acid requirement of the pig. This is contrary to the previously cited results of Yacowitz *et al* (1951) which clearly indicate that a relationship exists in chicks. The question arises, therefore, with respect to the present experiment, as to whether the level of vitamin B_{12} used was sufficiently high to demonstrate a relationship. As previously stated, vitamin B_{12} was added at a level of 25 μg. per pound to the appropriate rations as the Merck Supplement SP 622 containing 12.5 mg. of vitamin B_{12} per pound. This product is standardized by the L.L.D. (cup assay) method. However, this same product when assayed by the previously described method of Skeggs *et al.* (1948) using *Lactobacillus leichmannii* gives a value of 3 mg. of vitamin B_{12} per pound. If the latter assay procedure more nearly reflects the amount of vitamin B_{12} available to the pig it may well be that the levels used were not high enough to demonstrate a relationship between vitamin B_{12} and pantothenic acid.

Summary

Symptoms of pantothenic acid deficiency were produced in pigs fed a low protein (14 percent) ration containing corn, soybean oil meal, fish solubles and minerals. The addition of calcium pantothenate at a level of 4 mg. per pound of feed resulted in a very marked growth response.

The addition of calcium pantothenate to a higher protein (17.8 percent) ration, made up of the same feed constituents and containing the same level of pantothenic acid, resulted in only a slight increase in growth. However, this increase was not statistically significant.

The addition of vitamin B_{12} to both basal rations did not result in any significant growth response.

The data seem to indicate that higher levels of protein exert a sparing effect on the pantothenic acid requirement of the pig.

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RIBOFLAVIN DEFICIENCY IN BABY PIGS[1]

W. P. LEHRER, JR. AND A. C. WIESE[2]
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WINTROBE, Mitchell, and Kolb (1938) learned that symptoms of a vitamin B-complex deficiency in suckling pigs could be temporarily relieved by the administration of crystalline riboflavin. Hughes (1938, 1939) made similar observations upon pigs of a more advanced age. Wintrobe (1939) reported some growth response when suckling pigs on a vitamin deficient ration were given riboflavin.

Hughes (1940) found the minimum riboflavin requirement for pigs to be 1.0 to 3.0 mg. per 100 pounds of body weight. This determination was made on pigs weighing approximately 40 pounds at the beginning of the experimental period.

Wintrobe *et al* (1944) reported that riboflavin deficiency in young pigs was associated with growth impairment; rough, dry and thin hair coats; a mottled erythematous eruption together with scaling and ulceration of the skin; lens opacities; normocytic anemia; and abnormal gait. Krider, Terrill, and Van Pouche (1949) reported that 1.4 mg. of riboflavin per pound of ration appears to be the practical minimum for weanling pigs. Mitchell and associates (1950) report that for the growing suckling pig the estimated riboflavin requirement is 1.2 to 2.3 mg. per kg. of diet.

The trials reported here were undertaken to observe a riboflavin deficiency in the young suckling pig and to note the response to riboflavin supplementation.

Experimental

Two separate groups of Duroc pigs, 48 hours old, were used as experimental animals in both trials I and II. The four pigs in each

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[3] We wish to acknowledge the assistance of W. V. Hartwell and R. V. Dahlstrom who helped care for animals used in the experiment. Credit is due Dr. L. H. Scrivner and Dr. W. B. Ardrey for assistance with post mortem studies.

We are also indebted to Merck and Company, Rahway, New Jersey, through the courtesy of Dr. D. F. Green, for supplies of thiamin, riboflavin, pyridoxine, nicotinic acid, inositol, choline, p-Aminobenzoic acid. biotin, calcium pantothenate and tocopherol; to Lederle Laboratories Inc., Pearl River, New York, through the courtesy of Dr. E. R. L. Stockstad, for pteroyl-glutamic acid; to E. I. du Pont de Nemours and Company, New Brunswick, New Jersey, through the courtesy of Dr. S. Waddell, for crystalline vitamin D_3.

trial were litter mates. They were kept in individual metal cages 2.5 by 2.5 feet in size, equipped with wire mesh bottoms and underlying screens to facilitate the separation of urine and feces. The experimental diet was a "synthetic milk" having the composition given in table 1 and containing no riboflavin. The control animals were fed this ration plus 0.65 mg. riboflavin per kg. "synthetic milk". For complete details

TABLE 1. COMPOSITION OF RATION [1]

Component	Percent	Vitamins Added [3]	Mg. per Kg. Milk
Casein (Labco)	30.0	Thiamin	0.65
Cerelose	37.4	Calcium Pantothenate	1.30
Lard	26.6	Nicotinic Acid	2.50
Salts [2]	6.0	Inositol	26.00
		Choline	260.00
		p-Aminobenzoic Acid	2.60
		Pteroylglutamic Acid	0.052
		Biotin	0.01
		Pyridoxine	0.65
		Alpha-tocopherol	1.00
		2-methyl-1, 4-naphthoquinone	0.28
		Vitamin A	2,000 I.U./kg.
		Vitamin D	200 I.U./kg.

[1] The lard was homogenized with the solution of casein, cerelose and salts to produce an emulsion containing 13 percent solids.
[2] Modified salt mixture of Phillips and Hart (1935).
[3] Control animals also received 0.65 mg. riboflavin per kg. liquid diet.

concerning the preparation of this diet see Wiese, *et al* (1947). The feeding, care, and diet modifications for baby pigs were the same as that described by Lehrer *et al*, (1949).

Results and Discussion

Signs of nutritional deficiency became apparent within the first one to two weeks of these experiments. The animals failed to gain in weight and lost their appetite and normal appearance. Their hair coats and skin—at first clean, smooth, and sleek—became thin, coarse and unthrifty in appearance. The skin became dry and scaly and hair fell out readily. As the trials progressed, diarrhea and scours, prevalent in all deficient animals, became very severe and in some cases ultimatley resulted in rectal hemorrhaging and inflammation of the anus. Animals also exhibited an apparent sensitivity to light. All the above symptoms,

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[2] Departments of Animal Husbandry and Agricultural Chemistry.

[3] We wish to acknowledge the assistance of W. V. Hartwell and R. V. Dahlstrom who helped care for animals used in the experiment. Credit is due Dr. L. H. Scrivner and Dr. W. B. Ardrey for assistance with post mortem studies.

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trial were litter mates. They were kept in individual metal cages 2.5 by 2.5 feet in size, equipped with wire mesh bottoms and underlying screens to facilitate the separation of urine and feces. The experimental diet was a "synthetic milk" having the composition given in table 1 and containing no riboflavin. The control animals were fed this ration plus 0.65 mg. riboflavin per kg. "synthetic milk". For complete details

TABLE 1. COMPOSITION OF RATION [1]

Component	Percent	Vitamins Added [3]	Mg. per Kg. Milk
Casein (Labco)	30.0	Thiamin	0.65
Cerelose	37.4	Calcium Pantothenate	1.30
Lard	26.6	Nicotinic Acid	2.50
Salts [2]	6.0	Inositol	26.00
		Choline	260.00
		p-Aminobenzoic Acid	2.60
		Pteroylglutamic Acid	0.052
		Biotin	0.01
		Pyridoxine	0.65
		Alpha-tocopherol	1.00
		2-methyl-1, 4-naphthoquinone	0.28
		Vitamin A	2,000 I.U./kg.
		Vitamin D	200 I.U./kg.

[1] The lard was homogenized with the solution of casein, cerelose and salts to produce an emulsion containing 13 percent solids.
[2] Modified salt mixture of Phillips and Hart (1935).
[3] Control animals also received 0.65 mg. riboflavin per kg. liquid diet.

concerning the preparation of this diet see Wiese, *et al* (1947). The feeding, care, and diet modifications for baby pigs were the same as that described by Lehrer *et al*, (1949).

Results and Discussion

Signs of nutritional deficiency became apparent within the first one to two weeks of these experiments. The animals failed to gain in weight and lost their appetite and normal appearance. Their hair coats and skin—at first clean, smooth, and sleek—became thin, coarse and unthrifty in appearance. The skin became dry and scaly and hair fell out readily. As the trials progressed, diarrhea and scours, prevalent in all deficient animals, became very severe and in some cases ultimatley resulted in rectal hemorrhaging and inflammation of the anus. Animals also exhibited an apparent sensitivity to light. All the above symptoms,

with the exception of rectal hemorrhaging and inflammation of anal mucosa, were reported by Wintrobe, Buschke, Follis, and Humphreys (1944). These workers reported lens opacities after pigs had been on a riboflavin-deficient diet for 96 days. This eye abnormality may have been similar to the condition noted in animals reported in these trials.

An abnormal gait was noted after animals had been on the experimental diet for four weeks. A longitudinal cracking of feet was observed. The gaits of the pigs appeared stiff, mincing, and hesistant; at times

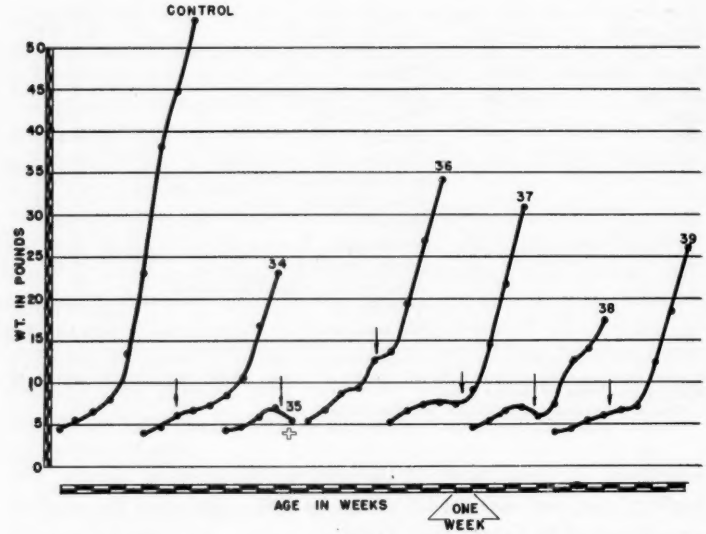

Figure 3. Growth rate of pigs on riboflavin deficient diet. The arrows indicate supplementation of riboflavin, the cross shows when pig 35 died.

they walked on the tips of the hoofs. These findings coincide with the report of Wintrobe, Buschke, Follis, and Humphreys (1944), who started pigs on a riboflavin deficient diet at 21 days of age.

The slow rate of growth (figure 3) and low dietary intake are consistent with the findings of Mitchell, Johnson, Hamilton, and Haines (1950).

Estimation of the urinary excretion of riboflavin with riboflavin-deficient animals was impossible because of continual scouring and subsequent contamination of samples with fecal material.

Figure 1 shows poor growth, unthrifty appearance and rough thin hair of pig 37 after being on the riboflavin deficient diet for 32 days. A comparison of pig 38 and its normal control litter mate receiving adequate amounts of riboflavin (0.65 mg. per kg. liquid diet) is presented in figure 2.

Trial I—The treatment of riboflavin-deficient pig 35 began on the 27th day. At the time of supplemental feeding, this pig was extremely

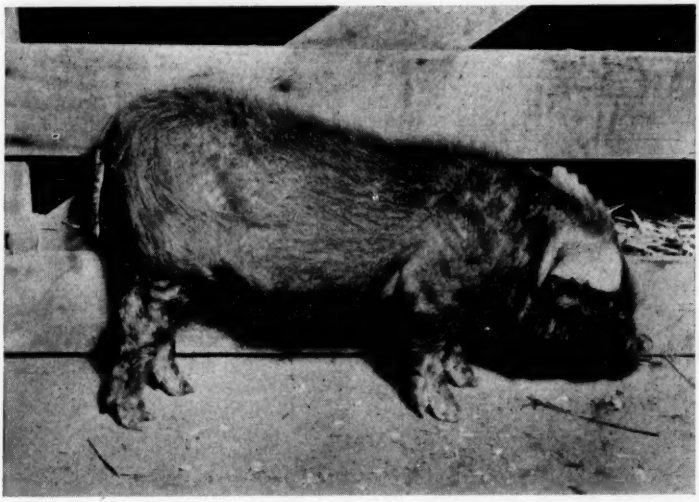

Figure 1. Pig 37 after being on riboflavin deficient diet for 32 days.

weak and refused to eat, thus force-feeding had to be practiced. This pig was fed 500 μg. of riboflavin daily up to the time it died on the 30th day.

The remaining two deficient pigs, 34 and 36, were fed 500 μg. of riboflavin beginning on the 31st and 33rd days, respectively. This supplementation was continued for 6 days. Following supplementation, appetites of both animals improved; scouring ceased and hair coats became more lustrous. A few days after discontinuing supplementation (46th day), however, both pigs exhibited recurrence of deficiency symptoms. On the 50th day, 500 μg. of riboflavin was fed; this amount was daily added to their dietary intake of liquid diet until termination of the trial on the 56th day. That these symptoms were not completely

corrected was possibly due to low level of riboflavin supplementation or the supplement was not fed for a sufficient period of time.

Macroscopic examination of these animals at the end of the trial revealed small size, scaly skins, and rough sparse hair coats. Necropsy showed necrosis and sloughing of the corium with hemorrhage in the proliferative germinal layers of the skin. The liver showed leucocytic infiltration and many polymorphonuclear leucocytes in the liver and

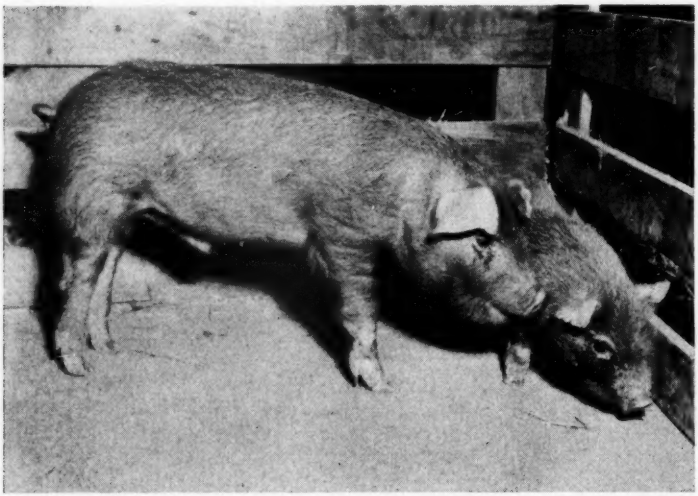

Figure 2. Companion of control Pig 33 on left, and litter mate riboflavin deficient Pig 38 on right, at 34 days of age.

blood. The kidneys exhibited a subacute glomerulonephritis and cloudy swelling with destruction of the tubular epithelium. The lungs showed pneumonia and microscopically there was leucocytic and erthrocytic infiltration, with many polymorphonuclear leucocytes present.

Trial II—When it became evident that the dosage of riboflavin described above was inadequate, this trial was undertaken to recheck the riboflavin deficiency symptoms observed in trial I, and to attempt to more completely alleviate the symptoms with larger supplementations of riboflavin.

Deficiency symptoms occurred in the same sequence and severity as did those in trial I. The supplemental feeding of riboflavin began on the 31st day when 1 mg. of riboflavin was fed to all deficient animals;

supplementation at this amount was continued for the following eight days. This amount of riboflavin gave immediate relief to all deficiency symptoms. However, immediately upon removal of riboflavin from the diet all animals again began scouring and vomiting. On the 48th day to alleviate these symptoms, and throughout the duration of this trial, pigs 37 and 39 were fed 1.5 mg. and pig 38 was fed 1 mg. per day of riboflavin, respectively.

At the termination of this trial, all pigs appeared to be normal in every respect except for their small size. Postmortem examination, however, revealed many abnormalities. The liver and kidneys showed blotchy and subcapsular microscopic hemorrhages. Microscopically, the liver showed a granulocytic infiltration of the interlobular spaces with intercolumnar congestion in the subcapsular region. There were many polymorphonuclear leucocytes in the blood. The kidneys showed an interstitial congestion with many polymorphs in the blood. The incomplete cure of the internal symptoms was probably due to the short duration of riboflavin supplementation.

Hughes (1940) using 80 pound pigs indicated the minimum riboflavin requirement for the growing pig was 1.0 to 3.0 mg. per 100 pounds body weight. From the studies reported above, the suckling pig apparently has a requirement nearer the maximum amount reported by Hughes (1940) than the minimum.

Summary

Riboflavin deficiency of baby pigs is associated with alopecia, anorexia, poor growth, rough hair coat, dermatitis, scours, ulcerative colitis, inflammation of anal mucosa, vomiting, light sensitivity, unsteady gait, and many abnormal internal complications.

The supplementation of 1 to 1.5 mgs. of riboflavin per day for 16 days cured the external symptoms. However, many of the internal tissue changes were not corrected. This may have been due to insufficient amounts of riboflavin being fed, or the supplementation was for too short a period of time.

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THE EFFECTS OF STILBESTROL IMPLANTS ON SWINE OF DIFFERENT SEXES[1]

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PREVIOUS studies have indicated that stilbestrol increases the growth rate and fat deposition of broilers (Thayer, Jaap and Penquite, 1945; Lorenz, 1945; Andrews and Bohren, 1947; Detwiler, Andrews and Bohren, 1950). Dinusson, Andrews and Beeson (1950) concluded that stilbestrol implants increased growth rate, efficiency of gain, and feed intake of growing and fattening heifers. Jordan and Dinusson (1950) reported that stilbestrol did not increase the growth rate of suckling lambs, but Perry, Andrews and Beeson (1951) obtained a significant increase in gains for lambs given stilbestrol implants while suckling their dams on pasture. Jordan (1950) reported that feeder lambs receiving stilbestrol made faster gains than the untreated animals. Braude (1950) concluded that barrows receiving iodinated casein and stilbestrol in the diet grew faster and utilized their feed more efficiently than animals on a control ration. Recent studies with swine (Dinusson, Klosterman and Buchanan, 1951; Woehling *et al.*, 1951) have indicated that stilbestrol has little effect on gains of growing-fattening pigs. Wallace (1949) observed that stilbestrol treatment of mature boars had little effect on semen production, but boars treated with stilbestrol implants prior to sexual development produced abnormal semen although sexual behavior was normal. Meites and Shay (1951) observed that vitamin B_{12} injections counteracted the growth inhibition observed in young rats fed stilbestrol.

This study was conducted to determine whether the use of stilbestrol implants for young growing swine of different sexes was advantageous as measured by rapidity of gains, efficiency of feed utilization, and carcass quality.

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Experimental Procedure

Experiment I

Thirty purebred Duroc pigs weighing approximately 35 pounds each were divided into six lots of five pigs each, with two lots of boars, two lots of barrows, and two lots of gilts. The pigs were placed on feed January 19, 1951, and fed in dry lot for 107 days. All pigs were self-fed the same ration (table 1) and had free access to water. The pigs were weighed weekly and records were kept on feed consumption. The results of experiment I are given in table 2.

One lot of pigs of each sex was kept as untreated controls while the other was given subcutaneous stilbestrol implants on the jowl. At the

TABLE 1. RATION USED [1]

Ingredients	Parts
Corn, ground	60.0
Soybean oil meal	28.0
Alfalfa meal	5.0
Meat and bone scraps (55% protein)	5.0
Bone meal	0.5
Limestone	1.0
Salt-trace mineral mixture [2]	0.53
	100.03

[1] One lb. of Lederle's Fortafeed, which contained 2,000 mg. of riboflavin, 4,000 mg. of pantothenic acid, 9,000 mg. of niacin, and 10,000 mg. of choline chloride, was added to each 400 lbs. of feed. Merck's Fuller's Earth B_{12} Concentrate was added at a rate equivalent to 1.25 mg. of vitamin B_{12} per 100 lbs. of feed.

[2] Salt-trace mineral mixture was composed of iodized salt 50 lbs., manganese sulfate 800 g., ferrous sulfate 398 g., copper sulfate 125 g., and cobalt carbonate 10 g.

beginning of the experiment and at the end of the first month the dosage was one 25 mg. pellet per pig, while at the end of two months two 25 mg. pellets were given per animal. The dosage was increased as it was noted that all external signs of estrus observed in the gilts following stilbestrol implantation disappeared prior to three weeks following treatment.

One boar in each of the two lots developed a severe case of dermatitis, which has been observed frequently at this station, and had to be removed from the experiment. One of the control barrows developed a serious scrotal hernia and was also removed.

At the close of the experiment all boars were slaughtered in the University meats laboratory, while the barrows and gilts were slaughtered

by a local meat packer. Cold carcass weights, with head on and leaf fat in, were taken after chilling for 24 hours at 36° F. Carcass yields were calculated on the basis of live weight at the end of the experiment and chilled carcass weight. Thickness of backfat was determined by averaging measurements taken at the first rib, last rib and last lumbar

TABLE 2. SUMMARY OF RESULTS OF EXPERIMENT I

	Lot 1 Control Boars	Lot 2 Stilbestrol Boars	Lot 3 Control Barrows	Lot 4 Stilbestrol Barrows	Lot 5 Control Gilts	Lot 6 Stilbestrol Gilts
Number of pigs [1]	4	4	4	5	5	5
Days on experiment	107	107	107	107	107	107
Av. initial wt., lbs.	36.0	34.6	35.2	34.8	35.3	33.3
Av. final wt., lbs.	224.9	194.5	190.0	186.5	186.6	181.8
Av. daily gain, lbs.	1.80†	1.50	1.46	1.43	1.43	1.40
Feed required per 100 lbs. gain, lbs	322	325	355	329	348	338
Av. dressing percentage [2]	75.26†	74.49	75.13	75.16	77.84	78.67
Av. thickness of backfat, inches	1.66	1.44	1.56	1.71	1.66	1.58
Av. carcass grade [3]	C-1	Med.	C-1	C-1	C-1	C-1
Av. palatability score [4]	3.86*	3.76	4.09	4.24	4.24	4.43
Av. shear reading, lbs.[5]	7.29	7.76	5.18	6.84	7.51	5.94
Av. weight of testicles, grams	546.92	573.13	.....	.....	.....	.....
Av. weight of ovaries, grams	.....	.....	.....	.....	4.42	4.21

* Significant at 5% level.
† Significant at 1% level.
[1] All lots had five pigs at start of trial.
[2] Dressing percentage $= \frac{\text{Chilled carcass wt. (shipper style)}}{\text{Final wt.}} \times 100$
[3] Carcass grade based on proposed government grades with following abbreviations:
Choice 1 — C-1 Medium — Med.
Choice 2 — C-2 Cull — Cull
Choice 3 — C-3
[4] Average palatability score is average of 11 and 12 judges' scores on roasts from four pigs in each lot. The basis of scoring is from 1 to 7 in order of increasing desirability.
[5] Shear readings were made on ½ inch cores and recorded in pounds to shear the core with three cores per roast and three shears per core.

vertebra. Weights were taken of the ovaries of the gilts and of the testes of the boars.

Wholesale loins were removed from the carcass of four pigs in each lot. Two roasts were removed from the loin adjacent to the ham from each carcass and placed in freezer storage at 0° F. until removed two to five days later for testing. Both roasts from each pig were roasted to an internal temperature of 185° F. in an electric oven at 350° F. Weights were taken before and after cooking. One roast was used for tenderness determinations with the Warner-Bratzler shear and the other was used for palatability tests.

The organoleptic tests were made on four consecutive days, using

four roasts each day. The judges were six girls, who had been carefully selected on a basis of threshold tests, and six staff members having previous experience on palatability committees. Ratings were made for aroma, flavor of fat, flavor of lean, and tenderness.

Experiment II

A total of 24 purebred hogs averaging approximately 84 pounds each were divided into three groups on a basis of weight, sex, and previous treatment. Each lot was composed of four barrows and four gilts. All animals were self-fed on good pasture for 73 days starting June 30,

TABLE 3. SUMMARY OF STATISTICAL ANALYSIS FOR EXPERIMENT I

(Sums of Squares)

Source of Variation	Degrees of freedom	Dressing percentage	Palatability Scores [1]	Thickness of back fat	Tenderness shear test [1]	Av. daily gain [2]
Total	23	131.72	3.12	0.61	47.81	(5) 0.1146
Sex	2	60.48**	1.16*	0.04	9.20	(2) 0.0691**
Treatment	1	0.19	0.03	0.03	0.21	(1) 0.0193
Sex × treatment	2	2.80	0.10	0.15	10.69	(2) 0.0262
Error term	18	68.25	1.83	0.39	27.71	.....

* $P \leqq 0.05$.
** $P \leqq 0.01$.
[1] Based on average value for each hog.
[2] Based on analysis of variance for disproportionate subclass numbers as the number of pigs varied between lots. The figures shown in parentheses are degrees of freedom used. The error term was calculated from within the sums of squares of different lots.

1951. Essentially the same ration was used as in Experiment I, except that the minerals were fed free-choice and no vitamin supplements were added.

The animals in lot 1 served as controls, those in lot 2 received a 50 mg. stilbestrol implant at the start of the trial but no further treatment, while those in lot 3 were given a 50 mg. implant at the start and at three-week intervals until the experiment was concluded.

One pig in lot 1 died during the experiment. Allowance was made for feed consumed and a calculated gain (Snedecor, 1946) was used in the statistical analysis.

The animals were slaughtered by a local packer at the end of the experiment. Average thickness of backfat was obtained as previously described and dressing percentage was determined on chilled carcass weights with the head off and leaf fat left in the carcass. Experiment II is summarized in table 4.

Experiment III

Twenty-four pigs averaging 92 pounds each were divided into two lots with seven barrows and five gilts in each. The same ration as used in Experiment II was self-fed on pasture starting September 13, 1951, for 63 days. The pigs in lot 1 were the controls, while those in lot 2 were given a 50 mg. stilbestrol implant at the start of the experiment and at the end of every three-week period until the trial was concluded.

TABLE 4. SUMMARY OF RESULTS OF EXPERIMENT II

	Lot 1 Controls	Lot 2 Stilbestrol 50 mg. at start	Lot 3 Stilbestrol 50 mg. every 3 weeks
Number of pigs per lot [1]	8	8	8
Days on experiment	73	73	73
Av. starting wt., lbs.	84.2	83.8	83.6
Av. final wt., lbs.	184.0	184.1	188.5
Av. daily gain, lbs.	1.31	1.37	1.44
Feed required per 100 lbs. gain, lbs.	384	466	367
Av. dressing percentage [2]	71.12	71.07	70.03
Av. thickness of back fat,[3] inches	1.78	1.71	1.81
Av. carcass grade [4]	C-1	C-1	C-2

[1] Four barrows and four gilts in each lot.

[2] Average dressing percentage $= \frac{\text{Chilled carcass wt. (head off—leaf fat in)}}{\text{Final wt.}} \times 100.$

[3] Average thickness of back fat from first rib, seventh rib, and last lumbar vertebra.

[4] Carcass grade based on proposed government grades:

Choice 1 — C-1 Medium — Med.
Choice 2 — C-2 Cull — Cull
Choice 3 — C-3

Results and Discussion

Sexual Behavior and Weight of Sex Organs. The stilbestrol treated gilts exhibited marked signs of estrus within 24 hours from the time of implantation. They also showed a marked restlessness, enlargement of the mammary glands and swelling of the external genitalia, which disappeared between two and three weeks following implantation. In the barrows there was considerable swelling of the rudimentary teats and, when allowed with the gilts, there was a marked tendency for riding, although examination of the genitalia after slaughtering gave no clues as to the cause. However, this phenomenon was observed in a rather high percentage of all barrows and was more evident shortly after stilbestrol treatment. Although there was some swelling of the rudimentary teats on the boars, no other differences were observed.

The ovaries of the gilts receiving stilbestrol were hyperemic and frequently had small cysts, which were more enlarged in the gilts receiving higher levels, while the control gilts were normal. Weights (Experiment I) of the ovaries averaged 4.42 grams for the control gilts and 4.21 grams for the stilbestol group. The average weight for the testicles of the boars (Experiment I) was 547 grams for the controls and 573 grams for the stilbestrol animals. There was more difference within lots than between lots, indicating little effect from the use of stilbestrol on weights of sex organs.

At the close of Experiment I, before slaughtering the animals, an

TABLE 5. SUMMARY OF STATISTICAL ANALYSIS FOR EXPERIMENT II

(Sums of Squares)

Source of Variation	Degrees of freedom	Dressing percentage [1]	Thickness of back fat [1]	Av. daily gain [2]
Total	21	79.57	0.773	(23) 0.380
Treatment	2	5.84	0.025	(2) 0.060
Sex	1	4.96	0.055	(1) 0.005
Subgroups	5	21.61	0.107	(2) 0.070
Error term	16	57.96	0.666	(18) 0.245

[1] Based on analysis of variance for disproportionate subclass numbers as one pig died in lot 1 and one was removed from lot 2 before slaughtering.

[2] Based on calculated value for pig missing in lot 1. Figures in parentheses are degrees of freedom used.

attempt was made to mate the boars receiving stilbestrol to normal gilts to check fertility and sexual behavior. All four boars exhibited normal libido and three were mated to three normal gilts that were available. Two of the gilts conceived and produced normal litters of eight and nine pigs. Although the third gilt failed to conceive, the mating was made during the early stages of estrus, which may have accounted for her failure to settle.

Average Daily Gains. In Experiment I the average daily gains were 1.80, 1.50, 1.46, 1.43, 1.43, and 1.40 pounds for lots 1 through 6, respectively. The increased gains made by lot 1, the control boars, were highly significant as compared to the other lots, while the differences between the other lots were not significant. The larger gains made by the control boars may be associated with a direct or indirect growth-stimulating action of the male sex hormones, as the boars receiving stilbestrol did not gain materially faster than the barrows or gilts.

In Experiment II the average daily gains amounted to 1.31, 1.37, and

TABLE 6. SUMMARY OF RESULTS, EXPERIMENT III

	Lot 1 Controls	Lot 2 50 mg. stilbestrol every three weeks
Number of pigs per lot [1]	12	12
Days on experiment	63	63
Av. starting wt., lbs.	91.8	91.9
Av. final wt., lbs.	193.5	192.0
Av. daily gain, lbs.	1.64	1.59
Feed required per cwt. gain, lbs.	423	457
Av. dressing percentage [2]	67.38	70.31**
Av. thickness of back fat,[3] inches	1.66	1.58
Av. carcass grade [4]	Choice #1	Choice #1

[1] Seven barrows and five gilts per lot.

[2] Average dressing percentage $= \frac{\text{Chilled carcass wt. (head off—leaf fat out)}}{\text{Final wt.}} \times 100.$

[3] Average thickness of back fat based on measurements at first rib, seventh rib, and last lumbar vertebra.

[4] Average carcass grade based on proposed government grades as shown in footnote 3, Table 2.

1.44 pounds for lots 1, 2 and 3, respectively. The differences, though slightly higher in the lots receiving stilbestrol, were not significant as measured statistically. Apparently there was no residual growth effect, as shown by the fact that lot 2, receiving only a 50 mg. implant at the start of the experiment, did not gain quite as fast as lot 3, which was given a 50 mg. implant every third week. The average daily gains in Experiment III were 1.64 and 1.59 pounds for lots 1 and 2, respectively, with the differences between lots not being statistically significant.

Feed per Hundred Pounds Gain. In Experiment I the amount of feed

TABLE 7. SUMMARY OF STATISTICAL ANALYSIS FOR EXPERIMENT III

(Sums of Squares)

	Degrees of freedom	Dressing percentage	Thickness of back fat	Av. daily gain
Total	23	230.20	1.10	2.54
Lots	1	51.39**	0.04	0.01
Sex	1	45.11*	0.02	0.28
Sex × lots	1	6.99	0.00	0.02
Subgroups	3	103.49	0.06	0.31
Error term	20	126.71	1.04	2.23

* $P \leqq 0.05$.
** $P \leqq 0.01$.

required for one hundred pounds gain ranged from 322 pounds for lot 1 up to 355 pounds for lot 3. The differences were small and apparently were largely due to feed wastage. The feed required per hundred pounds gain in Experiment II amounted to 384, 466, and 367 pounds for lots 1, 2 and 3, respectively. Since lot 1 (controls) and lot 3 (high stilbestrol) showed little difference, it appears likely that the higher feed requirement of lot 2 is associated with more feed wastage. The somewhat higher requirements per hundred pounds of gain in Experiment II as compared to Experiment I may be associated with the hot weather while this experiment was in progress. In Experiment III the feed required per hundred pounds gain was 423 pounds for Lot 1 compared to 457 pounds for lot 2. Although the feed requirements were somewhat higher than in previous trials, the difference may have been associated with somewhat more feed wastage.

Palatability Scores and Shear Readings. Average palatability scores (table 2) showed a definite preference for the meat of the barrows and gilts, with boars, both controls and those receiving stilbestrol, being graded lower. Average organoleptic scores gave a slight preference for the control boars over those receiving stilbestrol, indicating that stilbestrol was without effect on improving the eating qualities of the meat. Statistical analysis showed the preference for the gilts and barrows to be significant. During the cooking process the meat from all boars could be distinguished easily as it gave off the typical "boar odor."

The shear test revealed that differences in tenderness between lots were not significant but there was a highly significant correlation ($r = -0.723$) between the shear values and the palatability committee rating for tenderness. This indicates the reliability of the two methods of measuring tenderness.

Dressing Percentage and Thickness of Back Fat. In Experiment I the dressing percentages (table 2) were 75.26, 74.49, 75.13, 75.16, 77.84, and 78.67 for lots 1 to 6, respectively. Although the gilts (lots 5 and 6) dressed considerably more than the other lots, no difference in dressing percentage between control animals and those receiving stilbestrol was apparent. However, the higher dressing percentage of the gilts was highly significant when analyzed statistically. In Experiment II the differences in dressing percentages between lots were not significant. Although the gilts dressed 71.14, compared to 70.19 for the barrows, the difference was not significant. In Experiment III the pigs receiving stilbestrol (lot 2) dressed 70.31 percent compared to 67.38 for the controls, with statistical analysis showing the difference to be significant.

It appears likely that this difference was due to the allotment, since it did not show up in the previous experiments. However, the gilts dressed 70.47 percent compared to 67.69 for the barrows, with differences being statistically significant.

Summary

Three experiments were conducted to ascertain the effects of stilbestrol implants upon average daily gains, efficiency of gains, and certain carcass characteristics of growing-fattening swine of different sexes. Stilbestrol implants did not materially affect gains of either gilts or barrows but apparently caused a growth-depressing action upon young boars. The implants appeared to be without effect on the feed requirement for each hundred pounds of gain. The stilbestrol treated boars were fertile after having periodic implants made over a period of 107 days and manifested normal sexual behavior. Carcass studies indicated that stilbestrol implantation did not materially affect dressing percentage, thickness of back fat, carcass grade, or tenderness. The use of stilbestrol implants appeared to have little influence on organoleptic ratings of loin roasts and did not improve the eating qualities of meat from boars.

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THE USE OF CHROMIC OXIDE IN DIGESTIBILITY STUDIES WITH PIGS FED *AD LIBITUM* IN THE BARN[1]

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DIGESTION experiments carried out by conventional methods require either metabolism crates or feces bags to insure a quantitative collection. Furthermore, the daily feed intake has to be held constant. This is usually accomplished by restricting the allowance to a level lower than the voluntary feed intake of the animal if fed *ad libitum*. These prerequisites are prohibitive to the conduct of digestion experiments with large numbers of animals. It would be highly advantageous, however, to be able to conduct digestion studies with pigs during the time they are actually on a comparative feeding test. The question has also frequently been raised as to whether digestion secured on a restricted diet intake is comparable to that with *ad libitum* feeding.

The experiments reported herein were designed to test the applicability of the chromic oxide indicator method to digestion experiments with pigs under *ad libitum* barn feeding. This method has been shown to be useful in experiments with rats (Schürch, Lloyd and Crampton (1950)), with humans (Irwin and Crampton (1951)), and with sheep on rations which include ground concentrates (Crampton and Lloyd (1951)).

A. *Chromic Oxide indicator method vs. marker to marker collection in digestion experiments with pigs.* Four Yorkshire barrows averaging 150 pounds were confined to digestion crates and fed a commercial 14 percent protein hog finishing ration. A four-day preliminary feeding period was followed by a seven-day collection period during which 1 percent Cr_2O_3 was included in the diet by mixing it into the moistened feed just prior to each feeding. In the morning feeding of the first day, and in the evening feeding of the last day of the collection period, 10 grams of ferric oxide were added to the feed allowance for each pig as a marker for differentiating the feces belonging to the collection period. From the quantitative collection for the full marker to marker period, random feces samples of about one-fifth of the daily excretion from each pig were separately saved on the 4th, 5th and 6th days and

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analyzed for Cr_2O_3. Furthermore, samples of all morning collections and all evening collections were composited and analyzed for Cr_2O_3.

The digestion coefficients determined with the two different methods and from different samples are shown in table 1.

A statistical analysis of the data in table 1 indicates that there is no significant difference between the average values obtained by the Cr_2O_3 indicator method and the conventional marker to marker collection.

From these results the conclusion can be drawn that the Cr_2O_3 indicator method for measuring digestibility is as reliable as a seven-day marker to marker collection for swine.

TABLE 1. MARKER TO MARKER COLLECTION VS. Cr_2O_3 INDEX METHOD FOR DETERMINING DIGESTIBILITY IN PIGS

Method of Determination	% Digestibility of Dry Matter				
	Pig Numbers				
	1	2	3	4	Average
Marker to Marker Collection (7 days)	77	78	75	76	77
Chromic Oxide Method					
Random sample at 4th day	77	80	77	77	78
" " " 5th "	77	77	79	78	78
" " " 6th "	79	79	76	77	78
Average	78	79	77	77	78
Composited Morning Feces (7 days)	77	76	76	77	77
Composited Evening Feces (7 days)	76	76	76	75	76

B. *Digestibility under ad libitum feeding in a comparative feeding trial.* Sixteen fattening rations composed of different combinations of barley, oats, wheat and wild oats, plus a suitable protein-mineral supplement were fed in a feeding trial using four pigs per ration. For present discussion the plan of the trial is of no consequence excepting to note that all 64 pigs were penned individually in the regular piggery pens used for swine growth studies.

To get uniform distribution, the chromic oxide was mixed into a moist aliquot of the experimental diet in concentration to give a 1 percent level in the final ration. This premix was then passed through a meat grinder, dried, crumbled and mixed uniformly with the balance of the feed. For this experiment the preliminary and the collection periods each lasted six days.

In the barn the rations were fed *ad libitum*. The pigs weighed about 140 pounds each.

Paralleling this trial with the 64 pigs, four others were confined to digestion crates and digestibility determined during five successive periods for five of the 16 rations involved in the barn test. The rations selected were chosen to represent expected high and low digestibility. The object of this part of the study was to furnish a partial control for the barn test. The results are not strictly comparable since the same pigs were used with each of the five rations, and further because they were restricted to 4 pounds feed each per day while the barn fed pigs averaged nearly 7 pounds per day under *ad libitum* feeding.

The resulting digestion coefficients, all determined by the Cr_2O_3 method, are shown in table 2.

An analysis of variance applied to the results in table 2 did not reveal any significant differences between the values determined in the barn and those found using metabolism crates. The fact, however, that in one set of experiments, 64 different pigs were used, whereas in the other set all the experiments were carried out with the same four pigs brings in an unknown but systematic factor connected with time or age which could not be eliminated statistically. The figures do not indicate any important effect of this confounding but the data should not be taken as a critical test of the effects of restricted vs *ad libitum* feeding on digestibility. We believe the experiment does demonstrate the practicability of including a test of digestibility of rations as a part of a feeding trial under barn conditions.

C. *Tentative procedure for the determination of digestion coefficients with pigs fed ad libitum in the barn.* The findings discussed in the previous section suggests the following tentative procedure for the determination of digestibility under barn conditions.

The experimental animals are allotted at random to pens in the barn in accordance with the design of the feeding trial, and fed *ad libitum* the experimental diets containing approximately 1 percent chromic oxide (Cr_2O_3). (In order to avoid separation due to its high specific gravity, the chromic oxide is moist-mixed with a few pounds of the diet, using about half its weight of water. This premix may be pelleted by passing it through a meat grinder, drying the strings thus produced in the oven and then crumbling them for mixing into the experimental diet).

After a four-day preliminary period, a daily sample of 300–400 grams of well-formed and clean feces from each pig is collected from

TABLE 2. DRY MATTER DIGESTIBILITY OF 16 RATIONS DETERMINED DURING GROWTH TEST [1]

Ration No.	Lab. Control Test (metabolism cages)		*Ad libitum* Feeding in Growth Test (barn)		Ration No.	Lab. Control Test (metabolism cages)		*Ad libitum* Feeding in Growth Test (barn)	
	%		%			%		%	
1	77		82		9			77	
	77		82					76	
	77		82					81	
	77	77	84	82				78	78
2			81		10	78		81	
			80			78		81	
			81			79		78	
			80	80		78	78	79	80
3			79		11	78		78	
			76			78		74	
			81			79		78	
			82	80		79	79	82	78
4			78		12			78	
			80					76	
			80					73	
			77	78				77	76
5			78		13			76	
			77					80	
			77					80	
			74	77				77	78
6			72		14			74	
			70					79	
			70					73	
			76	72				74	75
7			77		15			74	
			78					75	
			..					79	
			73	76				74	76
8	74		70		16	73		71	
	72		76			72		72	
	74		76			70		70	
	72	73	79	76		73	72	72	71

[1] Individual values rounded to nearest percent; averages based on actual data and then rounded.

the floor of the pen for four successive days. The total four-day collection is dried at 105° C. The analysis of feed and feces for chromic oxide and for the proximate principle or nutrient in question, then permits the calculation of the respective digestion coefficients from the formula:

$$\% \text{ digestibility of nutrient} = \frac{100\ (a-b)}{a}$$

where a=parts of nutrient (e.g., dry matter, protein, ether extract, etc.) per unit index material in the food, and b=parts of nutrient per unit index material in the feces.

In the case that no individual pens are available, group penning may be employed for small groups. In order to be able to distinguish between the feces from each pig, different markers as ferric oxide, carmine, charcoal, etc., can be individually administered for the week of the test. From the differences in color, the feces from the different animals can be recognized

Summary

The chromic oxide method for determining digestion coefficients with pigs was shown to yield similar results as the conventional collection method.

A tentative procedure for the determination of digestion coefficients during comparative feeding trials with *ad libitum* barn feeding is described.

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THE MINIMUM REQUIREMENT OF YOUNG PIGS FOR A PURIFIED SOURCE OF CAROTENE[1]

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IN order to facilitate studies to determine the comparative value of various natural feeds as sources of provitamin A, it became necessary to know the minimum requirement of young pigs for a purified source of carotene. A review of literature relative to this problem revealed the need for further investigation of these requirements using criteria which are more dependable and sensitive to measure the state of vitamin A nutrition of the pig.

Braude *et al.* (1941) reported that one pig given crystalline carotene in oil showed a good rate of gain but no liver storage of vitamin A on 13.2 γ/kilogram body weight daily while administration of 39.6 γ to two pigs resulted in small liver reserves of vitamin A. Dunlop (1935) found that approximately 60 mg. of carotenoids from natural sources per 100 pounds of feed was sufficient to provide good growth, permit a slight storage of vitamin A in the liver, and prevent the appearance of deficiency symptoms. Although these requirements are based on feed intake, Lund (1938) on the basis of these figures, reported the mimimum carotene requirement for pigs to be 48 γ per kilogram body weight daily. Guilbert *et al.* (1937) reported the requirement of four pigs for crystalline carotene in oil to be between 26 and 33 γ per kilogram body weight daily when night-blindness studies and liver storage of vitamin A were used as the criteria of a vitamin A deficiency.

Mann *et al.* (1946) and Lewis *et al.* (1942) concluded on the basis of small animal experimentation that the blood concentration of vitamin A was a more sensitive criterion of a vitamin A deficiency than dark adaptation. Boyer *et al.* (1942), Grummer *et al.* (1948), and Eveleth *et al.* (1949) reported the use of blood plasma vitamin A levels as an index of the status of vitamin A nutrition of dairy calves, swine, and sheep respectively.

Moore and Sykes (1940) and Eveleth *et al.* (1949) have reported the use of cerebrospinal fluid pressures as a criterion for a vitamin A deficiency in calves and sheep.

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These experiments were undertaken to determine the minimum requirement of young pigs for purified carotene as measured by blood plasma vitamin A levels, liver storage of vitamin A, cerebrospinal fluid pressures, nightblindness studies, and rates of gain.

Experimental

This investigation consisted of two experiments. Each experiment was divided into two periods, a depletion period during which the pigs were fed a diet low in carotenoids in order to produce a vitamin A deficiency, and a supplementation period during which the pigs were given graded levels of crystalline carotene[3] in cottonseed oil in addition to the low-carotenoid diet. Supplementation was commenced when the blood plasma vitamin A levels of all pigs had dropped below 7 γ/100 ml. plasma. The crystalline carotene was dissolved in cottonseed oil and kept at a temperature of 34° F. The concentration of carotenes in the supplement was periodically determined by means of an Evelyn photoelectric colorimeter with a 440m.u. filter. The galvanometer reading was recorded and the concentration of carotenes determined by referring to a calibration curve prepared by plotting the percent transmission against concentration for a series of carotene[3] solutions of known concentration. The supplement was given to the pigs orally in gelatin capsules each day at noon. Feed and water were offered *ad libitum*. Blood plasma was analyzed for vitamin A content according to the method of Kimble (1939) and livers were analyzed for vitamin A content according to the method of Davies (1933). Cerebrospinal fluid pressures were observed by anesthetizing the pig by intraperitoneal injection of chloral hydrate and obtaining a pressure reading by lumbar puncture. This method has been described by Sorensen *et al.* (1951). Nightblindness studies were conducted as described by Hughes *et al.* (1937).

In experiment 1, 20 grade Hampshire pigs varying in age from two to three weeks were kept in raised, wire-floored cages in an unheated basement room and no attempts were made to control the environmental conditions. A low-carotene depletion ration consisting of hulled oats (groats) and cracked wheat, 85 percent; dried skimmilk, 15 percent; and irradiated yeast 0.4 oz. was fed throughout the depletion and supplementation periods. Fluid skimmilk was fed during the first few

[3] A purified crystalline carotene (80–85 percent beta form) derived from carrots. Purchased from Nutritional Research Associates, Inc., So. Whitley, Ind.

TABLE 1. RESPONSE OF PIGS TO VITAMIN A DEPLETION AND SUBSEQUENT CAROTENE SUPPLEMENTATION—EXPERIMENT 1

Carotene per kg. body wt.[1]	No. pigs	Av. da. gain[2]	Average plasma vitamin A			Av. liver vitamin A
			Initial	End of depletion period (8 wk.)	End of suppl. period[3] (10 wk.)	
		lb.	γ/100 ml.	γ/100 ml.	γ/100 ml.	γ/gm.
none	4	0.31	22.1	<5.0	<5.0	none
10 γ	4	0.60	28.2	<5.0	10.8 (±1.13)	trace
25 γ	4	0.71	24.5	<5.0	14.2 (±1.57)	0.13
40 γ	4	0.61	28.7	<5.0	19.3 (±0.06)	0.32

[1] Crystalline carotene in cottonseed oil administered orally in gelatin capsules each day.
[2] Average daily gain during supplementation period.
[3] Lot averages with standard error.

weeks. Following depletion, the animals were allotted for supplementation as follows: Lot 1, control lot which received no supplement, and lots 2, 3, and 4 which received 10, 25, and 40 γ purified carotene respectively per kilogram body weight daily.

In experiment 2, 20 grade Hampshire pigs, three weeks of age, were kept in metabolism cages in a room where the temperature was thermostatically controlled at a minimum of 70° F. The room was kept dry and

TABLE 2. RESPONSE OF PIGS TO VITAMIN A DEPLETION AND SUBSEQUENT CAROTENE SUPPLEMENTATION—EXPERIMENT 2

Carotene per kg. body wt.[1]	No. pigs	Av. da. gain[2]	Average plasma vitamin A			Av. liver vitamin A
			Initial	End of depletion period (7 wk.)	End of suppl. period[3] (7 wk.)	
		lb.	γ/100 ml.	γ/100 ml.	γ/100 ml.	γ/gm.
none	5	0.48	20.3	<5.0	<5.0	none
10.0 γ	5	1.04	23.8	<5.0	13.4 (±0.55)	trace
17.5 γ	5	1.19	23.2	<5.0	18.3 (±1.06)	trace
25.0 γ	5	1.23	19.5	<5.0	23.8 (±0.07)	0.39 γ

[1] Crystalline carotene in cottonseed oil administered orally each day.
[2] Average daily gain during supplementation period.
[3] Lot averages with standard error.

air was circulated by electric fans. An improved low-carotene depletion ration consisting of coarsely ground hulled oats, 79.5 percent; meat scraps, 10 percent; dried skimmilk, 10 percent; APF supplement,[4] 0.5 percent; and irradiated yeast 0.4 ounce was fed during the entire experimental period. Following the depletion period, these pigs were allotted for supplementation as follows: Lot 1, control which received no carotene, and lots 2, 3, and 4 which received respectively 10, 17.5, and 25 γ carotene/kilogram body weight daily.

Results and Discussion

The results of experiment 1 are shown in table 1. Initial blood plasma vitamin A values of all pigs were within the range of values usually observed in well-fed, healthy pigs as noted by Hentges *et al.* (1951). After eight weeks on the depletion ration, the levels of vitamin A in all pigs dropped below 7.6 γ/100 ml. plasma. Supplementation was begun at this point. During the supplementation period, a further decrease of blood plasma vitamin A was noted in the surviving pigs of lot 1 (control lot). The feeding of 10, 25, and 40 γ carotene/kilogram body weight daily resulted in plasma vitamin A values of 10.8, 14.2, and 19.3 γ/100 ml. plasma respectively. Each of these increases was highly significant statistically. No liver storage of vitamin A was observed in the pigs from lots 1 and 2. An average of 0.13 and 0.32 γ vitamin A per gram of liver was observed for the pigs of lots 3 and 4 respectively. No statistically significant differences were observed in average daily gains among the supplemented lots. An increase in cerebrospinal fluid pressure was evident when the blood plasma vitamin A levels had decreased to approximately 7 γ/100 ml. plasma, thereby being an early sign of injury caused by the vitamin A deficiency. Increased cerebrospinal fluid pressures were observed in all deficient pigs. Supplementation with carotene resulted in a gradual decrease of these abnormally high pressures. Further results of the cerebrospinal fluid studies may be found elsewhere (Hentges, 1951).

Although the method employed for detection of nightblindness was not entirely satisfactory, it was evident that nightblindness occurred only upon severe depletion of all vitamin A reserves, for example, no cases were noted until all blood plasma vitamin A values had decreased to immeasurable levels below 5 γ/100 ml. This finding is substantiated by similar reports by Lewis *et al.* (1942) and Mann *et al.* (1946).

A survey of the results of the several criteria used in experiment 1

[4] Lederle APF Feeding Supplement-5, Lederle Laboratories Division of American Cyanamid Co., Pearl River, N. Y.

resulted in the continued use of only blood plasma and liver concentrations of vitamin A. Under the comparatively severe environmental conditions of experiment 1, it appeared that 10 γ of carotene was sufficient for good rates of gain, 25 γ was needed for restoration of blood plasma vitamin A to initial levels, and 40 γ was adequate for liver storage of vitamin A.

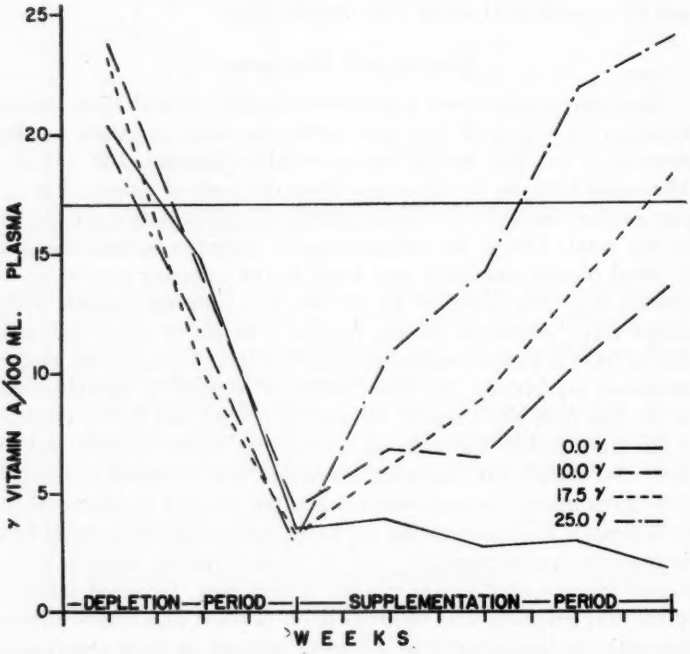

Figure 1. Effect of carotene supplementation on blood plasma vitamin A levels—Experiment 2.

The results of experiment 2 are shown in table 2. A clearer presentation of the average blood plasma vitamin A values is found in figure 1. Under the controlled environmental conditions of experiment 2, 25 γ of carotene/kilogram body weight daily was adequate for restoration of blood plasma levels of vitamin A in a five-week period and small liver reserves of vitamin A were also present at the end of the eight-week supplementation period. Daily feeding of 17.5 γ carotene/kilogram body weight did not permit measurable liver vitamin A storage but

the plasma vitamin A content was restored to its initial level after eight weeks. Ten γ carotene/kilogram body weight daily was again adequate for good rates of gain and protection against gross pathological changes but was inadequate for restoring the plasma vitamin A content to original values in an eight-week period.

The results of these two experiments indicate that 25 γ purified carotene per kg. body weight daily is the minimum amount required by young vitamin A deficient pigs for restoration of adequate blood plasma levels and small liver reserves of vitamin A.

These experiments point to the need for detailed descriptions of experimental procedures and conditions. The minimum requirement of young pigs for carotene apparently depends upon the environmental conditions, percentages of various carotenoids in the supplement, severity of depletion of experimental animals before supplementation, and most important of all, the criteria used to measure the state of vitamin A nutrition of the pig.

Summary

The minimum requirements of young pigs, which were depleted of their vitamin A reserves, for purified carotene (80–85 percent beta carotene) in cottonseed oil are reported. Criteria used were blood plasma and liver concentrations of vitamin A, cerebrospinal fluid pressures, nightblindness, and rates of gain.

To restore initial blood plasma vitamin A levels and provide some liver storage of vitamin A, 25 micrograms of purified carotene per kilogram body weight was shown to be the minimum daily requirement. The feeding of 17.5 micrograms per kilogram body weight daily did not permit liver storage of vitamin A but was sufficient to restore initial plasma vitamin A levels under the environmental conditions of experiment 2.

Ten micrograms of purified carotene per kg. body weight daily was sufficient to overcome gross deficiency symptoms and to promote good average daily weight gains.

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THE EFFECT OF THYROID ON CAROTENOID METABOLISM IN SWINE[1]

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IN recent years thyroprotein has been fed to swine in an attempt to increase the growth rate while thiouracil has been fed in attempts to increase the economy of meat production. Whether these drugs affect the metabolism of vitamin A in swine is unknown, but that adverse effects might exist is suggested by certain observations on rats. When the basal rate of the rat is increased, vitamin A disappears from the tissues at a faster rate than from normal tissues (Johnson and Baumann, 1948). On the other hand dietary levels of carotene that produce measurable stores of vitamin A in normal rats fail to do so in the presence of thiouracil (Johnson and Baumann 1947b; Kelley and Day 1948), and it has been suggested that thiouracil may interfere with the conversion of carotene into vitamin A. In the present study pigs were depleted of vitamin A and then were given either thiouracil or iodinated casein, and their subsequent responses to carotene or vitamin A determined.

Methods

The experiments were performed in four series in each of which comparable 10–14 week-old pigs of the Poland China or Chester White breeds from the Station swine herd were given the following ration ad libitum: oats, 40.5; wheat or barley, 40.5; soybean oil meal, 18; salt, 0.5; and lime, 0.5. In the last series 3 percent of skimmilk powder was added to the diet at the expense of the soybean oil meal.

Monthly analyses of the vitamin A in the blood plasma were made until the concentration in any series fell below 8 γ per 100 ml. The pigs were then divided into groups one of which served as the control, while the others received various levels of thiouracil or of thyroprotein (iodinated casein). In the first series these substances were fed only during the final period; in later series the feeding of these substances was begun 1–2 weeks before the end of the depletion period, and it

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station, Madison.

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was continued throughout the subsequent period of supplementation. The levels varied from series to series. Thyroprotein (3.32 percent thyroxin) at 0.1 percent of the diet caused a failure in growth and a severe diarrhea (series 1 and 2). In series 3, 0.05 percent was fed, while in series 4 the initial level was 0.04 percent for the first 11 days, followed by 0.02 percent for the final period. Thiouracil was fed at levels ranging from 0.1 percent of the diet in series 2 to 0.3 percent in series 4. The details are given in table 1.

The depleted pigs in the normal, hypothyroid, and hyperthyroid state were then fed either carotene or vitamin A for 2–3 weeks, and measurements were made of the growth rate and of the amounts of vitamin A in the blood, liver, and kidney. In some experiments analyses were also made of the adrenals, but these proved to be devoid of the vitamin. Certain pigs were given large supplements of carotene just prior to slaughter and analyses made of the vitamin A in the intestinal wall and in the lymph as well as in the blood, liver, and kidney.

The carotene supplement consisted of commercial carotene (83 percent β-carotene and 17 percent a-carotene) dissolved in cottonseed oil (Wesson) with an equal quantity of α-tocopherol. The vitamin A supplement was prepared by diluting halibut liver oil with cottonseed oil containing added α-tocopherol equal to the amount of vitamin A. These supplements were administered daily to the pigs by capsule or by calibrated syringe at the levels of 45 γ of carotene or 12 γ of vitamin A/lb. of body weight.

Samples of systemic blood were secured according to the method described by Carle and Dewhirst (1942). Lymph was obtained by cannulation at the first or second anastomoses of ducts running from the intestinal wall, but in later series lymph was secured in greater quantity by cannulation of the mesenteric lymph duct before its junction with the thoracic duct.

For the determination of vitamin A in plasma, the method of Kimble (1939) was modified for the analysis of 10 ml. of plasma which permitted the accurate measurement of about 7 γ of vitamin A/100 ml. of plasma. Lymph was analyzed in a similar manner except that alcoholic KOH was used in place of alcohol and the samples were saponified 30 min. before extraction.

Samples of liver, kidney, and small intestines, and the adrenal glands were analyzed colorimetrically for vitamin A and carotene (Dann and Evelyn, 1938; Johnson and Baumann, 1947a).

TABLE 1. GROWTH OF PIGS DURING VITAMIN A DEPLETION AND SUBSEQUENT RESPONSE TO SUPPLEMENTATION

	Depletion Period				Supplementation Period				
Series	No. of pigs	Initial weight[2]	Days depleted	Gain during depletion[1-2]	No. of pigs	Supplement after depletion	Days on suppl.	Days on carotene	Av.da. rate of gain
		lbs.		lbs./day					lbs.
1	4	24±4	48	.38±.08	2	none	—	21	.76
					2	.1% thyroprotein	21	21	.02
2	7	45±3	91–105	.80±.05	1	none	—	0	.64
					2	none	—	21	1.20
					2	.1% thyroprotein	23–30	21	—.55
					2	.1% thiouracil	23–30	21	.82
3	11	38±3	155–172	.80±.05	2	none	—	0	.97
					2	none	—	16	.89
					3	.05% thyroprotein	23–27	16	.29
					3	.2% thiouracil	27–30	16	1.43
4	11	34±1	97–111	.92±.04	2	none	—	19	1.35
					2	.04–.02% thyroprotein	27–28	19	.88
					2	.3% thiouracil	31	19	1.22

[1] Does not include the 2–3 weeks prior to supplementation.
[2] Mean ± standard error of the mean.

Results

Growth and plasma vitamin A during depletion and repletion

The average rate of gain during the depletion period was 0.8–0.9 pound/day in three of the four series (table 1). The time necessary for depletion varied from 48 days in series 1 to 155–172 days in series 3

TABLE 2. PLASMA VITAMIN A OF PIGS FED CAROTENE WITH THYROPROTEIN OR THIOURACIL

Series	Plasma vitamin A		Supplements	Days on carotene[1]	Final plasma vitamin A[2] γ/100 ml.
	Initial γ/100 ml.	Depleted γ/100 ml.			
1	26.6±3.1[4]	6.4±0.4[4]	——	21	35.4
			.1% thyroprotein	21	11.8[3]
2	20.4±1.1	6.1±0.8	——	0	6.9
			——	21	22.4
			.1% thyroprotein	21	15.6
			.1% thiouracil	21	22.6
3	22.8±2.1	8.4±0.5	——	0	9.6
			——	16	19.8, 30.4
			.05% thyroprotein	16	14.0
			.2% thiouracil	16	26.2
4	19.7±0.4	6.1±0.4	——	19	23.4, 18.2
			.04–.02% thyroprotein	19	14.4, 15.1
			.3% thiouracil	19	26.5, 41.5

[1] 45 γ of carotene/pound body weight/day.
[2] Value at 14 days.
[3] Individual values.
[4] Standard error of the mean.

(table 1), presumably because of variations in the initial stores of vitamin A in the pigs.

Thyroprotein depressed the growth rate in all series. When the dose was 0.1 percent of the diet the pigs either just maintained their original weights (table 1, series 1) or they lost weight (table 1, series 2). At lower levels of thyroprotein, 0.04–0.02 percent, some gain in weight was observed, 0.88 pound/day, as compared with 1.35 pound/day in the control animals (table 1, series 4). Growth responses to thiouracil were irregular: 0.1 percent appeared to depress growth somewhat (table 1, series 2); growth was essentially the same on 0.3 percent of the drug as

in the control group (series 4), while 0.2 percent of thiouracil appeared to increase the growth rate (series 3).

The administration of carotene to the depleted pigs resulted in normal levels of plasma vitamin A in the control animals and in those fed thiouracil (table 2), but in pigs fed thyroprotein the levels of plasma vitamin A were less than those in the other groups, e.g., in series 4 the average plasma vitamin A was 20.8 γ/100 ml. in normal pigs, but only 14.7 γ/100 ml. in the hyperthyroid group (table 2).

Storage of vitamin A in the tissues

The administration of carotene or vitamin A resulted in a significant accumulation of vitamin A in the tissues. In a typical animal which had received carotene for 16 days (table 3, series 3, pig 1), the vitamin A content of the liver was 7.35 mg. while that of the kidneys was 0.12 mg. (7.47 mg. total) as compared with an average total storage of only 0.66 mg. for all the unsupplemented animals. This result, incidentally, also illustrates the relative unimportance of the kidney for the storage of vitamin A in the pig; usually the liver contained from 10–200 times as much vitamin A as the kidneys. The amounts of vitamin A found in the tissues of the pigs fed carotene varied somewhat between individuals and also between series (table 3) but in general the amounts of the vitamin found in the livers and kidneys were approximately the same in the hyper- and the hypothyroid animals as in the normal controls. The average amounts of vitamin A accumulated by all the pigs fed carotene in the 4 series were 4.92 ± 2.08 mg. for the normal animals, 7.87 ± 4.7 mg. for the hyperthyroid pigs, and 6.97 ± 2.64 mg. of vitamin A for the hypothyroid animals (table 3).

When preformed vitamin A was fed to two normal and two hyperthyroid pigs, the amounts of vitamin A in the tissues were found to be 10.12 and 6.08 mg. in the normal pigs as compared with 9.25 and 6.66 mg. in the hyperthyroid animals (table 3, series 4).

Since the doses of carotene and vitamin A given were based on body weight, the total amounts administered per animal varied several-fold, for the weights of the animals at depletion were not uniform. It, therefore, appeared that possible differences due to metabolic rate might become more apparent if a correction were applied for inequalities in carotene intake. Results were calculated as "percentages of storage", defined as the mg. of vitamin A stored in the liver and kidneys x 100/mg. of carotene or vitamin A ingested during the period of supplementation. According to this method of expressing results the averages

for the four series were 8.49±3.89 for the hyperthyroid pigs, 5.16±1.70 for the normal animals, and 5.94±1.72 for those fed thiouracil. On this basis the differences between the hyperthyroid and normal pigs appear

TABLE 3. STORAGE OF VITAMIN A IN THE TISSUES OF PIGS UNDER DIFFERENT METABOLIC CONDITIONS

(Each value in the table refers to one animal)

Series	Supplement	Vitamin A in liver and kidneys of			Vitamin A in liver and kidneys of depleted and unsupplemented pigs
		Normal pigs	Hyperthyroid pigs	Hypothyroid pigs	
		mg.	mg.	mg.	mg.
1	Carotene	4.67 1.57	5.19 2.80		
2	Carotene	2.81 3.85	5.03 2.83	4.61 5.89	.96
3	Carotene	7.47 5.60	9.35 18.81 10.35	10.56 10.38 4.59	1.14 .32
4	Carotene	5.17 8.22	10.23 6.27	4.02 8.77	.21
Av. of 4 series		4.92±2.08[1]	7.87±4.70[1]	6.97±2.64[1]	.66
4	Vitamin A	10.12 6.08	9.25 6.66		

[1] Standard error of the mean.

to be greater than those represented in table 3 although they are still not significant statistically. The differences between the normal groups and pigs fed thiouracil tended to disappear.

Vitamin A in lymph

Under ordinary equilibrium conditions the vitamin A content of the mesenteric lymph ranged from 15.1–26.8 γ/100 ml. (table 4) and usually was very similar to that in the portal or systemic blood of the same animal.

A preliminary experiment showed that when halibut liver oil contain-

ing 5.6 and 7.5 mg. of vitamin A was fed 6 hours before slaughter, the amounts of vitamin A in the lymph rose to 67 γ/100 ml. compared with blood values of only 31.8–36.5 γ percent. This agrees with the view that lymph is the chief vehicle for the transport of the vitamin away from the intestine (Eden and Sellers, 1949).

TABLE 4. VITAMIN A IN THE BLOOD, LYMPH, AND INTESTINAL WALL OF PIGS BEFORE AND AFTER FEEDING LARGE DOSES OF CAROTENE

Metabolic state	Carotene fed 6–7 hrs. before slaughter		γ vitamin A/100 ml.			Vitamin A in intestinal wall
	mg./pig	γ/lb. B.W.	Systemic plasma	Portal plasma	Lymph	
			Before large dose of carotene			
Normal	0	0	25.4 (6)[1]	21.8	21.9	.09
Hyperthyroid	0	0	15.2 (3)	16.4	15.1	.03
Hypothyroid	0	0	25.1 (3)	24.1	26.8	.09
			After large dose of carotene			
Normal	34.6	450	26.7	27.8	449	.24
	47.4	225	21.1	33.5	249	...
	26.6	180	26.0	24.7	158	.27
Hyperthyroid	16.6	450	24.7	24.7	242	.14
	54.9	450	12.0	12.6	55.5	.15
	38.3	225	24.7	29.0	178	...
	43.4	225	29.3	28.7	98	...
	24.8	180	23.1	21.6	57.4	.16
Hypothyroid	45.0	450	22.1	31.8	218	.49
	38.7	225	30.6	31.5	124	...
	46.3	225	38.3	37.2	150	...
	23.4	180	52.0	48.8	156	.33

[1] Number in parentheses refers to number of animals.

The importance of the lymph was even more apparent when large doses of carotene were fed to approximately half of the animals in the metabolic series six to seven hours before slaughter: ten times the previous daily dose in the first two series, five times the daily dose in series 3, and four times the previous dose in series 4 (table 4). The amounts of vitamin A found in the lymph of the "unsupplemented" control pigs were very similar to those in their blood (table 4), and this similarity persisted in the presence of milky lymph due to the feeding of large amounts of cottonseed oil. However, when large doses of carotene were given, the amounts of vitamin A in the lymph were many times

those in the plasma and they were similar to the quantities of carotene fed per pound of body weight (table 4): e.g., a pig that had received 34.6 mg. of carotene (450 γ/pound body weight) had 449 γ of vitamin A/100 ml. of lymph as compared to only about 27 γ/100 ml. of the plasma; another pig receiving 180 γ of carotene/pound body weight had 158 γ percent of vitamin A in the lymph as compared to about 25 γ percent in the plasma. These single, large doses of carotene given just prior to slaughter did not alter the vitamin A content of the liver (Swick, 1949). These experiments confirm those of Thompson *et al.* (1947, 1949) and are in harmony with the view that carotene is converted to vitamin A in the intestinal wall of the pig. There was also more vitamin A in the intestinal wall of pigs fed carotene prior to slaughter, 0.25 γ of vitamin A/gm., than in the intestines of pigs with normal carotene intake: an average of 0.08 γ/gm. (table 4).

The amounts of vitamin A in the lymph of hyperthyroid or hypothyroid pigs after the ingestion of carotene were smaller than those in normal animals, but in every case the concentration in the lymph greatly exceeded that in the blood (table 4). No particular emphasis was placed on the results of the hyperthyroid group since the intestinal upset may have interfered with the absorption of the large, single doses administered.

Discussion

An abnormal metabolic rate can affect vitamin A metabolism in two ways: It can alter the rate of disappearance of the vitamin from the body and it can alter the mechanism for the conversion of carotene to vitamin A. When a hyperthyroid condition is established, the need for the vitamin increases, but in the rat at least, more vitamin A accumulates from a given amount of ingested carotene than in the normal rat (Johnson and Baumann, 1947b). In this experiment hyperthyroid pigs accumulated amounts of vitamin A at least as large as those in the normal animals; they appeared to be somewhat larger when expressed as the percentages of ingested carotene stored as vitamin A. It is possible that an enhancing effect on vitamin storage may have been masked by an intestinal upset with the hyperthyroid pigs absorbing less carotene than the controls. This possibility is suggested by the relatively low amounts of vitamin A found in the lymph of hyperthyroid pigs after the ingestion of large, single doses of carotene.

The hypothyroid pigs fed carotene stored essentially the same amounts of vitamin A in the liver as the control pigs, and the amounts of vitamin

A in their lymph ranged from 49 to 99 percent of that in control pigs fed corresponding large, single doses of carotene. Valid objections may, of course, exist to the feeding of thiouracil to pigs as a practical measure, but interference with carotene metabolism is not one of them, since the amounts of thiouracil fed in the present experiments were equal to or in excess of the quantities used in fattening swine. Furthermore, the duration of thiouracil treatment was only slightly less than that reported to be optimal for fattening.

Summary

1. Weanling pigs were depleted of vitamin A and were then rendered either hyperthyroid with iodinated casein or hypothyroid with thiouracil, and then were given carotene for 16–21 days. Essentially similar stores of vitamin A were found in the livers and kidneys of the 3 groups, with a suggestion of increased stores in hyperthyroid animals that were free from diarrhea.

2. When a large dose of carotene was given six to seven hours before slaughter, there was a marked increase (up to 20-fold) in the concentration of vitamin A in the mesenteric lymph and a much smaller rise in the blood plasma and intestinal wall. The results favor the view that carotene is converted into vitamin A in the intestinal wall of the pig.

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SWINE DYSENTERY: TREATMENT WITH 4-NITRO AND 3-NITRO-4-HYDROXY PHENYL ARSONIC ACIDS AND ANTIBIOTICS[1, 2]

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SWINE dysentery, a disease characterized by the presence of blood and/or mucus in the feces, was first recognized as a specific disease in swine by Whiting, Doyle and Spray (1921). Later Doyle (1944) isolated a strain of *Vibrio coli* from the intestinal mucosa of pigs with characteristic outward symptoms of the disease. When fed cultures of the organism, normal pigs developed the same symptoms after 27 days. More recently Salisbury, Smith and Doyle (1951) have reported the effect of aureomycin and streptomycin in treatment of the disease, and Boley and associates (1951) have reported the efficacy of arsanilic acid and bacitracin in controlling a natural outbreak of swine dysentery. Robinson (1951) used arsonic acid derivatives for the same purpose.

This communication describes some experiences with the use of arsenicals and antibiotics in the treatment of swine suffering a natural outbreak of the disease and of swine infected experimentally.

In the fall of 1950, swine dysentery was diagnosed in the Hormel Foundation herd of approximately 1500 swine of all ages. The breeding schedule in this herd is such that farrowing occurs the year around, at an average rate of a sow per day. All farrowing takes place in a large one-room barn containing 68 pens. The sows and litters are confined to this barn for 56 days, at which time they are transferred to a farm five miles away, where weaned pigs, boars, bred sows and young brood stock are kept. The weaned pigs are housed in a barn in groups of 20 to 30 pigs; from 200 to 250 head are in the barn at all times. The brood stock is housed in portable frame hog houses in lots adjacent to this barn. Weaned pigs that are not retained for breeding stock are transferred to a third farm where they are grouped in lots of 75 to 100. It is obviously difficult to control a communicable disease in a herd so managed that pigs of all ages are being transferred from one farm to another almost daily, and facilities are being used to capacity.

[1] Supported by grants from the Hormel Foundation, Austin, Minnesota, and Dr. Salsbury's Laboratories of Charles City, Iowa, who supplied the 3-nitro-4-hydroxy phenyl arsonic acid and the 4-nitro-phenyl arsonic acid.
[2] Hormel Institute publication No. 71.

Swine dysentery was seen in individual pigs soon after the first case was diagnosed in the fall of 1950, and about two months later it was present on all three farms, and in all age groups. A large number of animals showed a rapid loss in weight due largely to dehydration, and many weaned pigs and some sows succumbed to the disease.

Experimental

At the time of the outbreak, tests were being conducted on the effect of different levels of aureomycin as a vitamin B_{12} and antibiotic feeding supplement and 4-nitro phenyl arsonic acid (4-nitro PAA) on the growth of weaned pigs. Pigs in control lots were the first to show characteristic symptoms of swine dysentery while pigs receiving the highest level of the antibiotic or arsenical were the last. For example, control pigs in the test with 4-nitro PAA became ill in the eighth week of the test, while those receiving 0.01 percent of the arsenical became ill two weeks later, but none of the pigs in the lot receiving 0.02 percent of the arsenical developed the disease over an 11-week period. However, one week after the arsenical was removed from the diet, some pigs in the latter group showed symptoms of the disease. These results indicated that 4-nitro PAA had a prophylactic effect on naturally occurring swine dysentery.

To test the effectiveness of the 4-nitro PAA and a related compound, 3-nitro-4-hydroxy phenyl arsonic acid (3-nitro-4-hydroxy PAA) in the treatment of this disease, a group of pigs was experimentally infected with material from sick pigs, which was prepared as follows: The colons of 1 to 3 sick pigs were removed immediately after slaughter, and ground in buffered saline in a Waring blender; the mixture was then filtered through a single layer of cheese cloth. The filtrate was made up to volume sufficient to provide a 10 cc drench for each test pig, which was given on the first day of each experiment. The material that did not pass the filter was mixed with a small portion of feed and placed in the feeders for the test lots.

All test pigs were from the Hormel Foundation herd and were fed a basal diet of common feedstuffs (Carpenter, 1951). The experimentally infected pigs were kept in 8 x 10′ pens with 10 x 12′ outside runways. The pens had concrete floors and were separated by a wire fence. They were cleaned at least four times daily.

When the arsenicals were administered through the drinking water, they were first dissolved in water using sufficient sodium or potassium hydroxide to bring the pH to about 7.

Aureomycin was added to feed in the form of a vitamin B_{12} and antibiotic feeding supplement containing 1.5 mg. of vitamin B_{12} and 1.8 gm. of aureomycin per pound; when applied as a drench, pure aureomycin hydrochloride was used. Both materials were supplied by Lederle Laboratories Division of the American Cyanamid Company.

Penicillin was added to the feed as a vitamin B_{12} and antibiotic feeding supplement containing 12.5 mg. of vitamin B_{12} and 2 gm. procaine penicillin per pound; when the procaine penicillin was administered orally, it was given in capsule form as a product having 80 percent activity. Both products were supplied by Merck & Co., Rahway, N. J.

TABLE 1. THE EFFECT OF TREATMENT WITH 3-NITRO-4-HYDROXY PHENYL ARSONIC ACID AND 4-NITRO PHENYL ARSONIC ACID ON PIGS EXPERIMENTALLY INFECTED WITH SWINE DYSENTERY

Lot No.	Treatment	Number of pigs	Pigs with symptoms[1]	Average gain per pig	
				10 days	20 days
				lb.	lb.
1 (not infected)	None	10	6[2]	0.1	2.0
2 (infected)	None	10	8	2.0	4.0
3 (infected)	400 mg. 3-nitro[3]	10	2	7.6	14.0
4 (infected)	800 mg. 4-nitro[3]	10	0	6.3	13.0

[1] Pigs showing outward symptoms of dysentery during first 10 days.
[2] One of the six died on the eighth day.
[3] Per gallon of drinking water for 10 days.

Bacitracin was added to the feed as an antibiotic feeding supplement containing 3 gm. of bacitracin per pound, supplied by U. S. Industrial Chemicals, Baltimore, Md.

Terramycin was added to the feed as an antibiotic feeding supplement containing 5 gm. of terramycin per pound. It was supplied by Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

Results

Experiment 1. Four lots of ten pigs each were treated as described in table 1. It is evident from the results (table 1) that the uninfected controls (lot 1), as well as all other pigs had been exposed to the disease prior to being placed on test. Two pigs in lot 3 developed bloody dysentery five days after being placed on test but no symptoms were evident on the tenth day. None of the pigs in lot 4 developed symptoms of the disease during the ten-day treatment period. The

disease occurred in both lots 3 and 4 between the tenth and 20th day; but the symptoms were alleviated by resuming medication. The average weight gain for the four lots over the 20-day period is an index of the protection given by the two arsenical compounds.

Experiment 2. In experiment 1 both the 3-nitro-4-hydroxy PAA and the 4-nitro PAA reduced the incidence of bloody dysentery in experimentally infected pigs, but after the medication was discontinued the pigs contracted the disease. The second test was conducted to compare the effectiveness of the two compounds when given at the same level, and to determine whether the disease could be prevented from recurring by continuous medication with 4-nitro PAA in the feed. For this test,

TABLE 2. THE EFFECT OF TREATMENT WITH 3-NITRO-4-HYDROXY PHENYL ARSONIC ACID AND 4-NITRO PHENYL ARSONIC ACID ON PIGS EXPERIMENTALLY INFECTED WITH SWINE DYSENTERY

Lot No.	Treatment		Number of pigs	Pigs with symptoms[3]	Average gain per pig	
	Water[1]	Feed[2]			10 days	20 days
					lb.	lb.
1	None	None	8	8	−1.0	−0.2
2	3-nitro	"	8	2	2.0	12.0
3	4-nitro	"	8	1	4.0	16.0
4	4-nitro	4-nitro	8	0	2.5	10.0

[1] 400 mg. per gallon of drinking water for 10 days.
[2] 0.025% in feed for 20 days.
[3] Pigs with outward symptoms of dysentery during first ten days.

four lots of eight weaned pigs each were infected as described above and given the treatments listed in table 2.

All the control pigs became infected during the first 10-day period and three pigs died during the 20-day period; eventually all pigs in this lot succumbed to the disease or became so emaciated they had to be killed. Of the two pigs in lot 2 that showed symptoms during the ten-day medication period, one recovered and the other was given 1000 mg. and 400 mg. of the 3-nitro-4-hydroxy PAA as a drench on the ninth and tenth days respectively. There were no symptoms of the disease in any of the pigs in lot 2 on the 11th day. During the last ten days of the test, three pigs in this lot developed swine dysentery; two were given 300 mg. of the 3-nitro-4-hydroxy PAA as a drench and recovered.

In contrast, one pig in lot 3 developed symptoms of the disease during the ten-day treatment period; the symptoms disappeared within 24 hours and no other cases occurred during the 20-day period. None

of the pigs in lot 4 developed outward symptoms of the disease during the entire test period. However, some of the pigs showed symptoms of toxicity from the arsenical; that is, a choppy gait, sensitivity to touch, and inability to get up readily.

Experiment 3. This test was conducted to determine the effect of a combination of 4-nitro PAA and the antibiotics procaine penicillin and bacitracin on the incidence of swine dysentery in experimentally infected pigs. Again four lots of ten pigs each were infected as described and treated as listed in table 3.

All the pigs in the control lot developed outward symptoms of bloody dysentery during a 21-day period; seven pigs developed the

TABLE 3. THE EFFECT OF TREATMENT WITH A COMBINATION OF 4-NITRO PHENYL ARSONIC ACID AND PENICILLIN OR BACITRACIN ON PIGS EXPERIMENTALLY INFECTED WITH SWINE DYSENTERY

Lot No.	Treatment		Number of pigs	Pigs with symptoms[3]	Average gain per pig	
	Water[1]	Feed[2]			7 days	21 days
1	None	None	10	10	1.2	0.3[4]
2	4-nitro	Bacitracin	10	2	6.0	20.9
3	"	Penicillin	10	2	4.5	20.7
4	"	Penicillin and 4-nitro	10	1	1.8	8.6

[1] 400 mg. of 4-nitro per gallon of drinking water for seven days.
[2] 6 gm. of bacitracin or procaine penicillin per 100 pound feed as antibiotic feeding supplement and 0.025 percent of 4-nitro compound.
[3] Pigs with outward symptoms of dysentery over 21-day period.
[4] Average gain for 14 days only.

disease during the first ten days of the test. On the 14th day, the pigs in lot 1 were divided into two additional lots (A and B). Lot A served as controls and received no medication, while the pigs in lot B received 500 mg. of 4-nitro PAA on the 14th day as a drench and were given drinking water that contained 400 mg. of the arsenical per gallon, for seven days. Six days later none of the treated pigs in lot B had a diarrhea while all but one of the untreated control pigs had swine dysentery. The pigs in lot A gained a total of 17 pounds during this seven-day test period compared to 39 pounds for the pigs in lot B; the week previous, the pigs in lot A gained 17 and those in lot B lost nine pounds.

One pig in lot 2 developed bloody dysentery 24 hours after the pigs were infected but the symptoms disappeared within 48 hours. Another

pig manifested the symptoms of bloody dysentery on the 12th day that continued through the 15th day. All other pigs appeared normal.

In lot 3 one pig developed bloody dysentery 24 hours after infection, but the symptoms disappeared in 24 hours. On the 14th day, one pig had a diarrhea with a trace of blood. On the 15th day all pigs appeared normal. No other symptoms were noted throughout the test period.

One pig in lot 4 developed bloody dysentery 24 hours after the start of the test; the diarrhea ceased on the third day. After the tenth day all stools indicated that the pigs were constipated. However, the treatment produced toxic symptoms in three pigs on the 20th day, and therefore the test was discontinued.

TABLE 4. THE EFFECT OF TREATMENT WITH 4-NITRO-PHENYL ARSONIC ACID FOLLOWED BY AUREOMYCIN AND TERRAMYCIN ON PIGS EXPERIMENTALLY INFECTED WITH SWINE DYSENTERY

Lot No.	Treatment		Number of pigs	Pigs with symptoms[3]	Average gain per pig	
	Water[1]	Feed[2]			7 days	56 days
					lb.	lb.
1	None	None	8	4	2.0	41
2	700 mg. 4-nitro	None	8	0	3.0	46
3	600 mg. "	Terramycin	8	1	4.8	64
4	500 mg. "	Aureomycin	8	0	4.9	63

[1] Per gallon of drinking water for seven days.
[2] 6 gm. terramycin or aureomycin per 100 pound feed as antibiotic feeding supplements after the first seven days.
[3] Pigs with outward symptoms of dysentery over seven-day period.

Experiment 4. This test was conducted to determine the effect of different levels of 4-nitro PAA in the drinking water for one week on the incidence of bloody dysentery. After a 7-day treatment, the diets of the pigs in lots 3 and 4 contained 6 gm. of terramycin or 6 gm. aureomycin as antibiotic feeding supplements per 100 pounds of feed respectively.

Fifty percent of the pigs in the control lot (table 4) developed outward symptoms of swine dysentery during the first seven days of the experiment and all pigs developed symptoms during the 8-week period. In general, the pigs in this lot were unthrifty and inactive.

Some of the pigs in lot 2 had a diarrhea during the first three days of the test period but no mucus or blood was seen in the stools. Although medication was discontinued on the seventh day, none of the pigs

developed a diarrhea until the 14th day and blood appeared in the stool on the 18th day; all pigs developed diarrhea during the eight-week period, and one pig died.

In contrast, one pig in lot 3 developed bloody dysentery the second day of the test, which disappeared within 24 hours, and none of the pigs had a diarrhea on the seventh day. None of the pigs in this lot developed symptoms of swine dysentery during the ensuing seven weeks. The pigs were kept in the same pens and on the same diet for an additional four weeks, and remained in apparent good health.

Three pigs in lot 4 developed diarrhea during the first three days of the test, but no blood or mucus appeared in the stools. All pigs had formed stools on the seventh day. During the last seven weeks, two pigs developed bloody dysentery; one case lasted approximately three days and then cleared up without further treatment whereas the other case required additional treatment.

Treatment of Naturally Occurring Cases of Swine Dysentery. Immediately after swine dysentery had become a herd problem, attempts were made to cure the disease in individual pigs as well as in groups. Pigs fed diets containing 2 gm. of aureomycin as an antibiotic feed supplement per 100 pounds of feed did not develop symptoms of the disease as readily as pigs fed the same diet without the antibiotic. In a preliminary experiment, two weaned pigs with swine dysentery were given 0.5 gm. of pure aureomycin as a drench on two successive days, and were fed a diet containing 20 gm. of aureomycin as an antibiotic concentrate. The symptoms disappeared, and the disease did not recur. However, when suckling pigs with swine dysentery were drenched on two successive days with 0.5 gm. pure aureomycin, the symptoms were not entirely eliminated. In other experiments, 200,000 units of procaine penicillin and 100,000 units of penicillin G sodium given orally to suckling pigs weighing 20 to 25 pounds eliminated the symptoms. However, neither penicillin nor aureomycin prevented recurrence of the disease after medication was stopped.

Outward symptoms of dysentery in suckling or weaned pigs were often eliminated within 24 hours after applying a single drench with either 3-nitro-4-hydroxy PAA or 4-nitro PAA. Occasionally a second drench was required, and a few cases that did not respond to the arsenical treatment did respond after one treatment with penicillin. Approximately 100 mg. of arsenical were used for each 10 pounds of pig weight. During the past year, more than 1500 suckling and

weanling pigs have been drenched with the arsenicals, with satisfactory results. About 200 sows with swine dysentery have been drenched with a solution of 3-nitro-4-hydroxy PAA (1.5 gm./400 pound pig weight) with satisfactory results; the few cases that did not respond were treated with approximately 1,500,000 units of penicillin, and the symptoms then disappeared.

The outward symptoms of the disease were eliminated from pigs drenched with 4-nitro PAA (100 mg./10 pounds pig). When either of the two arsenicals were given intraperitoneally, symptoms of the disease ceased, but severe toxicity or death resulted in some cases. Several lots of pigs were treated successfully with 400 mg. of arsanilic acid per gallon of drinking water for either a seven or ten day period. None of the above treatments prevented recurrence of the disease after treatment was stopped. Consequently, it was necessary to retreat individuals, as well as lots of pigs, and symptoms of toxicity (Carpenter, 1951) were noted in some pigs after repeated treatment.

One of the major problems involved in treating swine dysentery in a continuous swine-raising operation is that of medicating suckling pigs. Weaned or adult pigs can be treated through the drinking water or feed, but young suckling pigs must be force-medicated.

In an attempt to eliminate the disease from the Hormel Foundation herd, a program of rigid sanitation was tried, together with medication of all pigs regardless of age. All adult stock was given water containing 400 mg. of either 3-nitro-4-hydroxy PAA or 4-nitro PAA per gallon for a period of seven days. Aureomycin was added to all diets at a level of 6 gm. per 100 pounds of feed as an antibiotic concentrate. All suckling pigs and their dams were treated on two successive days with a drench of 3-nitro-4-hydroxy PAA (100 mg./10 pound pig weight for suckling pigs and 1.5 gm. per 400 pound sow). All animals showing diarrhea were isolated and all equipment used by caretakers was disinfected both before and after being used in the isolated pens. After one week of this program, the disease was still present. Therefore all dams and their litters were retreated; one half of the dams and their litters were treated again with 3-nitro-4-hydroxy PAA, and the other half received one treatment with procaine penicillin orally. All suckling pigs were given 480,000 units and sows 4,800,000 units. After another two weeks, the number of cases was reduced, but the disease was still present. It was also reappearing in a few pigs in the weaned pig herd.

Discussion. The experiments described above demonstrate that 3-nitro-4-hydroxy PAA and 4-nitro PAA, when administered through the drinking water, can prevent or eliminate outward symptoms of swine dysentery, that is, a diarrhea with blood and mucus in the stool. However, when medication is discontinued, under the environmental conditions imposed in the Hormel Foundation swine herd, the disease readily recurs. A combination of medication through the water with 4-nitro PAA and antibiotics in the feed produced satisfactory results under our test conditions, but a toxic condition resulted after about three weeks of medication.

Although the treatment did not completely eradicate disease from the herd, it has almost completely eliminated deaths from swine dysentery. Also, although a high percentage of the suckling pigs have contracted the disease, the weaning weights have increased during the past year.

A vibrio closely resembling the one described by Doyle (1944) is present in fecal material and colon contents of affected pigs. A spirochete which may be associated with the disease is also present. When pigs are treated with either of the arsenicals or penicillin or aureomycin, the spirochetes may entirely disappear or remain present in only small numbers. The vibrio, however, can usually be found, though in greatly reduced numbers. Feces from swine that have been treated and apparently cured still contain vibrio, indicating that although the symptoms of the disease are eliminated, the animals may still harbor organisms that can cause the disease.

Summary

The arsenicals, 3-nitro-4-hydroxy phenyl arsonic acid and 4-nitrophenyl arsonic acids have a prophylactic and curative effect on swine dysentery when administered through the drinking water. Also both arsenicals and the antibiotics, aureomycin and penicillin, eliminate the outward symptoms of the disease when given orally. No treatment used prevented the recurrence of the disease.

A combination of a water medication with 4-nitro-phenyl arsonic acid and one of the antibiotics—aureomycin, terramycin, penicillin or bacitracin—as well as the 4-nitro phenyl arsonic acid in the feed was the most effective treatment in curing and preventing the recurrence of the disease under the environmental conditions imposed.

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SOME PHYSIOLOGICAL CAUSES OF GENETICALLY DIFFERENT RATES OF GROWTH IN SWINE

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THE rates at which economic characters express themselves in domestic animals are determined by the genotype, the environment, and the interaction of these two factors. It has also been generally assumed that the genes governing expression of economic characters do so by controlling the rates at which glands concerned with growth or reproductive functions secrete the hormones specific for these effects. No study was found in the literature which was concerned with quantitative as well as qualitative analysis of the causes which underlie observed, genetically different rates of physiologic functions. Using two genetically different lines of Hampshire swine which differed greatly in their ability to gain weight and were available for study, such an analysis was attempted and is reported here. These two lines had been selected for slow and rapid rates of gain, for eight and nine generations, respectively, and at the time of this experiment the difference in weight between these two lines at 180 days of age was about 62 pounds (table 1).

That part of the study which was concerned with endocrine causes of the different rates of gain of the two lines included comparisons of pituitary and thyroid weights and the amounts of growth hormone contained in pituitaries from animals of both lines at four different ages and at 225 pounds or over ("mature").

In addition to the physiologic studies to be reported here, a series of nutrition experiments (Baird 1951) was conducted and will be summarized to characterize the two lines more completely. In a 72-day feeding trial, rapid-line pigs consumed significantly more feed daily (2.5 vs. 1.1 pounds), the daily gains of the two lines being 0.94 and 0.31 pounds respectively. Slow-line pigs required 3.64 pounds and rapid-line pigs 2.76 pounds of feed to produce one pound of gain. No significant difference was found between the two lines in the percentage digestibility of all nutrients, in their ability to metabolize gross energy, in the amount of heat production per square meter of body surface, or

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in the amount of metabolic nitrogen excreted per kilogram of ration consumed. It should be noted that, although these differences were not significant, the rapid line exceeded the slow line in all of these comparisons. The differences were significantly in favor of the rapid line in the amount of crude fiber digested and in the net-nitrogen requirements for maintenance. Of great interest was the observation that rapid-line animals, when pair-fed with slow-line animals, passed feed through the digestive tract at a significantly faster rate than did the slow-line animals (23.6 ± 2.3 vs. 34.8 ± 4.2 hours).

TABLE 1. PROGRESS IN SELECTION FOR RAPID AND SLOW RATES OF GAIN. (BODY WEIGHTS AT 180 DAYS OF AGE)

Generation	Rapid Line	Slow Line
0	141.8	141.8
1	140.8	126.8
2	161.1	151.6
3	139.9	107.6
4	129.2	106.5
5 (4s)[1]	164.5	133.6
6 (5s)	158.4	113.9
7 (6s)	113.5	80.9
8 (7s)	150.0	116.0
9 (8s)	171.8	110.0

[1] The slow-growing line was one generation behind the rapid line because the pigs of the fourth generation of the slow line were not numerous or thrifty enough to be used for breeding.

Experimental

Animals from the rapid and slow lines of both sexes were killed at the ages of 56, 75, 115 and 154 days, and at "mature" weight, the slow-line animals then being older because they required longer to reach that weight.

The pituitaries were collected within 30 minutes of the animal's death, identified and immediately frozen. Just before they were to be used for assay they were thawed, freed from adhering connective tissue and the pituitary stalk and weighed. Weights were also obtained of the anterior lobe alone, which was then macerated between two glass plates, dried in a calcium-chloride desiccator and ground to a fine powder. The drying and grinding of the glands was done not more than 48 hours before they were to be used for assay.

The thyroids were cleaned while fresh, weighed and discarded.

Assay Methods

Three criteria were used for assaying the growth-hormone content of the pituitaries: (1) urinary nitrogen retention, (2) increase in body weight, and (3) width of the epiphyseal cartilage. The assay animals were female white rats, hypophysectomized at 21 days of age. By 12 days after the operation the urinary nitrogen became relatively constant. After that time urine was collected from each rat separately for a three-day pre-injection, a four-day injection, and a one-day post-injection period. The urine collected from each rat during each of these periods was pooled and analyzed for its nitrogen content by the micro-Kjeldahl method. Changes in nitrogen excretion were expressed as a percentage of the nitrogen excretion during the pre-injection period.

In several preliminary trials crude anterior pituitary powder from pigs of the two lines was injected at levels varying from 2 to 10,000 gamma per day in an attempt to determine the optimum dose for each of the three criteria. Fifty gamma per rat per day was found to be optimal, permitting variations in hormone potency of individual glands from the two lines to express themselves to a maximal extent by all three tests. In subsequent assays this amount of powder was injected daily, in two equal doses each suspended in 0.5 ml. of distilled water, the injections continuing for four days. Thus a total of 200 gamma of pituitary powder was injected into each rat.

The rats were weighed daily during the whole test and until 24 hours following the last injection. They were then promptly autopsied, and the right tibia was removed and processed according to the method of Evans *et al.* (1943) to determine the width of the epiphyseal cartilage.

Since not all of the 56 pituitaries could be assayed simultaneously, they were assayed in series, in each of which the glands were paired by line, sex and age and represented as complete a spread of the five ages as could be arranged in view of the unequal number of pituitaries available in the age groups. Each gland was assayed on two rats. After it was shown that there was no significant difference between the series, the results were pooled.

Results

Comparison of pituitary and thyroid weights of 86 pigs of the two lines at the five different ages showed that there was no significant difference between the sexes except at maturity, when castrated males

had significantly heavier pituitaries. For this reason the gland weights were pooled by sex except for mature animals. On an absolute basis the pituitaries of the animals from the rapid line were heavier at 56, 75, 115 and 154 days of age, but this is because the rapid-line pigs were heavier (table 2). When expressed in proportion to body weight, these differences disappeared or were of doubtful significance. The same relationship holds for the thyroid gland weights (table 2).

The assay of 56 individual anterior pituitaries for growth-hormone potency indicated that the nitrogen retention method, while capable of detecting the presence of growth hormone (compare controls and treated groups; table 3), was not sufficiently sensitive for our purpose. In contrast the cartilage-width method and the body weight increases were highly sensitive. It was found that at all ages and weights a given weight of the rapid-line anterior pituitary powder contained more somatotrophic hormone than did the same weight of low-line powder. These differences were not significant for the 56 day-old pigs, perhaps because of small numbers, but were significant at all other ages and at maturity (table 3). The differences in growth hormone potency between the sexes were not significant and the data were therefore pooled by sex.

According to Marx *et al.* (1942, 1944) the nitrogen retention test and the cartilage test to a large extent measure the growth hormone potency of the pituitary, and are only slightly affected by thyrotrophic hormone. On the other hand the body weight increase of the test animals would be greatly modified by the synergistic action between growth and thyrotrophic hormone. In this study crude anterior pituitary powder was used which, in addition to growth hormone, contained appreciable amounts of thyrotrophic hormone. Hence the cartilage test measured primarily the growth hormone, and the body weight increase measured the potency of the powder for both hormones.

Discussion

Inspection of the data presented (tables 2 and 3) suggests several generalizations. The pituitaries and thyroids of the two genetically different lines do not differ significantly in weight if the difference in body weight at the ages at which these comparisons are made are taken into consideration; that is, the differences observed (table 2) are not line differences in organ weights but merely in total body weight.

As in all assays involving the estimation of the amounts of various hormones present in the pituitary, the question arises as to what is being measured by the assay: the actual rate at which a hormone is

TABLE 2. AVERAGE AGE, BODY WEIGHT, AND GLAND WEIGHTS BY AGE GROUPS FOR SLOW-LINE AND RAPID-LINE PIGS, WITH STANDARD ERRORS

Line	No.	Age in days	Body weight lbs.	Pituitary weight mg.	Anterior pituitary weight mg.	Thyroid weight g.	A.P. weight / Body weight $\times 10^3$	Thyroid weight / Body weight $\times 10^6$
S	2	57	15.5± 2.5	32.5±18.0	21.5±10.7	0.58±0.18	34±21	80±13
R	2	56	30.0± 2.0	54.9± 9.8	39.8± 8.0	1.39±0.02	40±4	102±6
R-S			14.5± 3.2*	22.4±20.5	18.3±13.4	0.81±0.18*	6±21	22±14
S	5	74	16.6± 2.0	26.7± 6.2	17.6± 2.9	0.94±0.22	24±4	126±24
R	5	75	35.6± 2.5	50.8± 9.7	35.7± 4.7	1.50±0.12	23±4	94±8
R-S			19.0± 3.2**	24.1±11.5	18.1± 5.5*	0.56±0.25	−1±6	−32±25
S	6	115	55.5±10.1	82.8±16.6	53.6±10.9	2.53±0.34	21±3	106±10
R	6	116	58.0± 8.5	116.3± 8.5	77.2± 6.1	3.71±0.66	31±3	138±11
R-S			2.5±13.2	33.5±18.7	23.6±12.5	1.18±0.74	10±4*	32±15
S	8	155	54.8± 7.0	108.9±11.8	62.3± 5.9	2.92±0.37	29±3	123±14
R	8	156	107.1± 5.1	127.9±10.3	96.9± 8.7	5.18±0.74	19±2	108±14
R-S			52.3± 8.7**	19.0±15.7	34.6±10.5**	2.26±0.83*	−10±4*	−15±20
S Males	16	264	223	223.0±11.1	159.1±11.5	(7)[1] 7.10±0.61	16±0.8	(7)[1] 72±5
R Males	16	232	222	213.0±10.7	154.4± 7.7	(6)[1] 8.14±0.70	15±0.7	(6)[1] 83±7
R-S				−10.0±15.4	−4.7±13.8	1.04±0.93	−1±1.1	11±9
S Females	6	231	221	161.3±20.1	108.3±11.2	6.98±0.56	11±1.2	70±5
R Females	6	219	222	113.2±15.9	82.1±10.7	(5)[1] 7.46±0.67	8±1.2	(5)[1] 72±7
R-S				−48.1±25.6	−26.2±15.5	0.48±0.87	−3±1.7	2±9

* Significant at the 5% level.
** Significant at the 1% level.
[1] Numbers in parentheses are numbers of pigs for which measurements were made and are given wherever different from the number entered in column two.

being secreted, the amount of hormone stored in the gland or the residual hormone which remains after secretory activity ceases. While no direct information on this subject has been obtained in this work, the close correlation between the growth rate of the pigs and the potency of their glands at different ages, suggests that the actual rate

TABLE 3. BIOASSAY OF ANTERIOR PITUITARIES. AVERAGES AND STANDARD ERRORS BY AGE AND LINE. STANDARD ERRORS OF DIFFERENCES TAKE ACCOUNT OF PAIRING

Assay Animals

Line	Age, days	Number	Cartilage width micra	Body weight gain grams	Nitrogen changes %
S	57	2	173.2± 2.4	3.8±0.5	18.6± 2.0
R	56	2	216.3± 4.3	5.0±1.0	13.8± 0.5
R-S			43.1± 2.0*	1.2±1.5	−4.8± 1.5
S	74	5	205.5±10.6	6.0±0.6	(2)[1] 37.1±20.5
R	75	5	250.6±15.9	7.1±0.7	(2)[1] 39.4±13.0
R-S			45.1±12.8*	1.1±0.3*	2.4± 7.5
S	115	6	197.0±11.7	5.3±0.7	(4)[1] 18.3± 9.2
R	116	6	241.3±14.4	6.6±0.4	(4)[1] 30.5± 7.9
R-S			44.3±11.5*	1.3±0.4*	12.2± 1.9**
S	155	8	198.6± 8.1	5.2±0.3	(3)[1] 24.7± 2.0
R	156	8	243.4±15.6	6.9±0.4	(3)[1] 27.6± 6.7
R-S			44.8± 9.9**	1.7±0.7*	2.9± 4.9
S	Mature	7	205.4±12.0	5.5±0.6	(4)[1] 21.5± 7.2
R	Mature	7	245.6± 7.8	6.7±0.4	(4)[1] 26.8± 4.0
R-S			40.2±10.0**	1.2±0.4*	5.3± 9.6
Control rats			(13) 134.7± 5.7	(17) 2.1±0.4	(17) 2.3± 4.3

* Significant at the 5% level.
** Significant at the 1% level.
[1] Numbers in parentheses are numbers of pigs for which assay measurements were made (except in last line, where they are numbers of rats used), and are given wherever different from the number entered in column three.

of growth hormone production and release are being measured (See also Robinson and Nalbandov 1951 for further evidence).

When comparisons of the growth hormone content of equal weights of anterior pituitary tissue from the two lines were made, it was found that rapid-line pituitaries contained significantly more growth hormone than did the slow-line pituitary tissue (table 3). This difference holds for all ages. It is interesting that, with the possible exception of the 56-day old pigs, the hormone potency per unit anterior pituitary tissue within each line remains quite constant with age, as evidenced by the

almost identical total increases produced in both the cartilage width and the body weight of the assay animals injected with pituitaries from pigs at 75, 115 and 154 days of age and at maturity. Consequently the differences in hormone potency between the two lines also remain constant throughout growth (40–44 units for cartilage growth and 1.1–1.4 grams for body weight of rats; table 3).

This suggests that at all ages, even at maturity, a given unit of anterior pituitary tissue secretes a constant quantity of growth hormone which, per unit pituitary tissue, does not increase during the active growth phase of the animal or decrease when maturity is approached

TABLE 4. COMPARISON OF THE TWO LINES FOR GROWTH HORMONE CONTENT CORRECTED FOR BODY WEIGHT AND PITUITARY WEIGHT. TOTAL HORMONE CONTENT PER UNIT LIVE WEIGHT

Age of donors days	Adjusted cartilage width (micra) $\times 10^6$		Adjusted weight gain (Kg) $\times 10^8$		Adjusted Nitrogen (percent) $\times 10^7$	
	Slow Line	Rapid Line	Slow Line	Rapid Line	Slow Line	Rapid Line
56	13	33	58	116	55	46
75	17	27	94	115	84	85
115	13	33	67	140	34	87
115	19	21	90	91	65	48
Mature	11	16	51	64	29	34

and reached. Observed differences in growth hormone potency between two animals are due to their different genetic constitutions.

Since the rate of growth hormone secretion appears to be constant at all ages the question arises how to account for the rapid growth rate observed in most animals just prior to the time when growth stasis is reached at maturity. For this reason it became of interest to determine the changes which occur in the growth hormone production if both the pituitary weight and the body weight of the animal are taken into consideration. This was done by performing the following recalculation of the data given in tables 2 and 3.

$$\frac{(\text{Average experimental value-average control value}) \times \text{Average ant. pit. Wt.}}{\text{Average body weight of donors}}$$

These recalculations of the data again show the difference in the growth hormone production between the two lines (table 4). In the rapid line, by all three criteria used, there is an increase in the total growth hormone production per unit of living animal from 56 to 115 days of age. This coincides with the most rapid rate of growth of this

line. There is a significant drop in total hormone production at 154 days of age which becomes still more pronounced at maturity. Pigs from the slow line reach a peak of growth hormone production on or before 75 days of age, maintain this level until 154 days and also show a significant drop at maturity (table 4).

These relations permit speculation on the causes of the growth stasis which occurs in animals approaching maturity. It may be unnecessary to postulate that the production of growth hormone is inhibited by pubertal hormones or similar checks. Since the ratio of anterior pituitary weight to body weight generally decreases with increasing age (table 2), it can be postulated that growth occurs only so long as the pituitary is able to maintain an adequate concentration of growth hormone in the body as a whole. Since the production of hormone per cell remains unchanged with increasing age of the animal (table 3), the only way in which hormone production can be stepped up is by increasing pituitary weight. However, since pituitary size does not increase in proportion to body size (table 2), the amount of circulating growth hormone falls gradually until it is below the level necessary for growth. Increasing body weight leads to increasing dilution of circulating growth hormone until, at the time the animal reaches mature weight, growth stasis results because the amount of hormone elaborated is then capable only of maintaining the attained size without further true growth. If this theory were correct one would expect to find a correlation between pituitary weight and body weight in relation to age. This was not found in the two lines studied possibly because of the fact that the animals involved can not be regarded as growing at rates typical of an unselected population. In our sample of the two lines, the rates of growth were quite irregular, several of the individuals lost weight, failed to gain or gained at a very low rate during periods when regular weight increases should have been obtained.

It appears probable that differences between the two lines in their ability to grow at slow and rapid rates are largely due to the significantly different amounts of growth hormone being secreted by them.

Summary

The growth hormone content of anterior pituitaries from 56 pigs belonging to two genetically different lines selected for rapid and slow rates of gain was assayed using hypophysectomized female rats. These indices of growth hormone content were used: ability to retain nitrogen, width of the epiphyseal cartilaginous disk and increase in body weight

of test animals. Powder from individual anterior pituitaries was injected for four days at the rate of 50 gamma per day.

The nitrogen retention test was found to be least reliable and most variable as an index of growth potency, highly repeatable results being obtained by the other two methods.

In relation to body weight, pituitary and thyroid weights of pigs from the two lines were not found to be significantly different.

Anterior pituitaries from rapid-line pigs at all ages contained significantly higher amounts of growth hormone per unit tissue than did the same amount of tissue from the slow line. There were no significant differences between sexes.

Each line contained the same amounts of growth hormone per unit tissue at all ages.

In relation to anterior pituitary size and body weight the amounts of growth hormones contained per unit of living animal, increased in the rapid line from 56 to 115 days of age and then decreased until mature size was reached. In the slow line this amount reached a plateau where it remained from 75 to 154 days of age and dropped in the mature animal. These relations probably account for the growth stasis observed in all animals at maturity.

The larger amounts of growth hormone consistently secreted by the rapid line at all ages of comparison accounts satisfactorily for the more rapid rate of gain observed in that line.

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SELECTION FOR FERTILITY IN TWO INBRED LINES OF SWINE[1]

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FOUNDERS of many of the breeds of livestock are credited with success in improving the merit of the breeds while engaging at the same time in the use of inbreeding. It has been supposed that the early breeders were very careful in selecting breeding animals and were thus able to avoid serious losses of merit in the breeds. Much interest in the effect of selection (American Society of Animal Production, 1951) has been evidenced by those in charge of the development of inbred lines of swine. Particular emphasis has been given to the importance of high fertility because it was recognized at an early date that a successful inbred line would have to reproduce in numbers sufficient to provide surplus breeding animals for use in crossing.

Laben and Whatley (1947) have already reported a study in an inbred line of Durocs in which replacements came from litters that averaged 1.2 pigs larger than average. Size of litter declined from 6.7 pigs in the first generation to 6.0 pigs in the fifth generation. Kottman, Lush, and Hazel (1948) calculated the theoretical improvement expected in each of several Poland China lines, taking into account the selection differentials, heritability estimates, and the effects of inbreeding. The annual selection differential for size of litter at birth was .25 pig; for size of litter at weaning it was .5 pig. In general the levels of performance at the time of their report were below those which would be expected as determined from the theoretical improvement due to selection less the decline in performance due to inbreeding.

The present study analyzes the records of two inbred lines of swine, which, in contrast to those studied by Laben and Whatley (1947) and Kottman, Lush, and Hazel (1948), were developed from crossbred foundations. The purposes of this study are to determine (1) how much selection was practiced for litter size at birth and for litter size at weaning and (2) how well the selection explains the performance of the

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lines, giving due attention to estimates of the effect of inbreeding on the traits.

Materials and Methods

The data for this study were taken from the spring farrowing records of the Minnesota No. 1 and the Minnesota No. 2, two inbred lines developed in the Minnesota swine breeding project conducted in cooperation with the Regional Swine Breeding Laboratory of the U.S.D.A. The Minnesota No. 1 was developed from a Danish Landrace-Tamworth crossbred foundation (Winters, Comstock and Dailey, 1943). The Minnesota No. 2 descended from matings between two Poland China inbred lines and the Yorkshire (Winters *et al.*, 1948). A detailed description of the method of gathering and recording the data and of the procedure and managerial practices employed in the development of all Minnesota inbred lines is given by Winters *et al.* (1947).

In analyzing the data the first step was to determine how much selection was practiced for the number of pigs farrowed alive and for the number of pigs weaned. The amount of selection was measured by the selection differential, *s*, in each trait in each line.

The second step, using the average selection differential for each character, the heritability of the trait, and the rate of increase in inbreeding, was to calculate the theoretical rate of change per year in the trait concerned. Symbolically, the theoretical rate of change per generation in phenotypic merit (Comstock and Winters, 1944) is

$$y=bI+sH$$

where y is the expected change per generation in the trait

b, the average change in the trait per unit of inbreeding (I) when $sH=O$

I, the increase in inbreeding per generation

s, the selection differential

H, the heritability estimate of the trait

The formula may be modified to give the theoretical rate of change per year, m, by (1) changing I to mean the increase in inbreeding per year and (2) dividing the term sH by G, which is the average interval between generations. The formula then becomes,

$$m=bI+\frac{sH}{G}$$

The third and last step was to compare the theoretical change in merit, m, with the actual change measured by the regression of performance in the trait on a time scale in units of years.

For use in the formula, estimates of *H* were taken from a recent study of data from the farrowing records of the various inbred lines in the Minnesota swine breeding project, including the Minnesota No. 1 and No. 2. (Cummings, Winters and Stewart, 1947.) The study yielded an estimate of approximately 22 percent as the heritability of litter size at birth. The estimate was corrected to the basis of a random bred population. As noted by Comstock and Winters (1944) the heritability of a trait in a closed population decreases as inbreeding increases, the decrease being approximately proportional to (1—F) where F is the inbreeding coefficient. The average inbreeding in the two lines for the period covered by this study was about 23 percent for the Minnesota No. 1 and 19 percent for the Minnesota No. 2. The corresponding heritability estimates for litter size for the two lines would be 17 and 18 percent respectively.

A heritability estimate of about 32 percent for the size of the litter at weaning was found in the Minnesota data studied by Cummings, Winters and Stewart (1947). When this figure is reduced to the basis of the average amount of inbreeding in each of the lines, *H* becomes 25 percent and 26 percent for the No. 1 and No. 2, respectively.

The value of *G*, which is the average age of the parents when their offspring are born (Dickerson and Hazel, 1944), or the average interval between generations, was found to be 1.62 years for the Minnesota No. 1 and 1.52 years for the Minnesota No. 2. The value of *I*, the average annual increase in the inbreeding coefficient, was 3.2 and 3.1 percent for the No. 1 and the No. 2, respectively.

The calculations of Comstock and Winters (1944) indicated that litter size would decline —.037 pig per litter for each increase of 1 percent in inbreeding in the dam and litter. Accordingly, *b*, the effect of inbreeding on litter size at birth, was set at —.037 pig per litter.

In the Minnesota No. 1 the intra-year, intra-sire regressions of number of pigs weaned per litter on inbreeding of dam and on inbreeding of litter were —.092 and —.063 respectively; the corresponding partial regressions were —.083 and —.047, indicating that for each 1 percent increase in the inbreeding of both dam and litter a decline of —.130 pig weaned per litter would be expected. As there was no comparable estimate derived from the No. 2 data, —.130 pig per litter was used as the value of *b* in this line also.

Values of *s* were computed separately for each line. For litter size, records of the number of live pigs farrowed were adjusted to the basis

of the age of 12 months for the dam by applying the corrections of table 1. These corrections were developed from the data of both lines by computing the intra-sire, intra-year regressions of number of live pigs farrowed on the age of the dam and the age of the dam squared, according to the method of Snedecor (1946).

Generally, in any one year, three age groups of females were available for selection. At weaning time certain sows, i.e. yearlings and two-year-olds, were selected for farrowing in the next spring. No three-year-olds were selected. Young females from the current year's farrow were also selected. These young females, usually in the majority, plus the

TABLE 1. CORRECTIONS APPLIED TO FARROWING RECORDS OF DIFFERENT AGE-GROUP FEMALES

Age of dam, months	Corrections to number of live pigs farrowed
9	+1.0
10	+ .6
11	+ .3
12	0
13	− .3
14	− .6
15	− .8
16	−1.0
22–27	−1.6
32–40	−1.0

yearlings and the two-year-olds made up the three age groups of females among which selection was practiced.

To get an estimate of the selection differential for litter size in the selected yearlings and two-year-olds the differences between the selected individuals' records and the herd average were averaged. The differences were weighted according to the number of female offspring which each selected individual raised to weaning age the following year. The steps in computing the average selection differential in one season required looking forward to the following year to determine which females actually had offspring available for selection.

In the case of the young female which was selected before she had farrowed a litter, the farrowing record of her dam was used. The maximum heritability of the trait when selection is based on the dam's record would be only half that when selection is based on the individual's own record. If the full value of H is to be applied, then the differential

in the individual should be halved; therefore, half the difference between the dam's record and the herd average was used as the selection differential in the young female, the differential being weighted according to the procedure above.

As a check on the preceding method of computing s, another set of values for s for litter size at birth was computed by using only the gilt record of the selected female (or that of her dam when the selected female had no record). The gilt record was compared with the gilt herd average of the year in which the gilt farrowed. The difference was weighted according to the procedure already described. Half of the difference between the dam's gilt record and the contemporary gilt herd average was used as the differential in the young female which was selected before she had made a record. When a female was reselected, the one difference between her gilt record and the contemporary gilt herd average was simply carried forward for use each time the female was selected. All selection differentials were in terms of gilt performance. This second set of values of s for size of litter at birth resulted in average values of s in females of .78 pig and .54 pig for the Minnesota No. 1 and No. 2, respectively. These values are to be compared with .82 pig and .65 pig for the No. 1 and No. 2, respectively (table 2). The values were in reasonably close agreement with those in table 2 where age correction factors were used in an attempt to adjust farrowing records to the common age of dam of 12 months.

As there is considerable computational labor in attempting to find correction factors and since the second set of values of s in terms of gilt litter size at birth agreed reasonably well with those where age corrections were attempted, the second procedure was used in computing values of s for pigs weaned. The values of s in terms of gilt performance in pigs weaned per litter may be found in table 2.

For boars values of s for litter size (table 2) were based on the average differential in the boar's dam, each of her age-corrected records being compared with the herd average of the year in which the record was made. The differential thus obtained for each boar's dam was halved to give an estimate of the differential in the boar, as in the case of the young selected female which had never farrowed. The average selection differential in the boars selected one year for use as sires of the next spring farrow was determined by weighting the differential in each boar by the number of his daughters available for selection the following year.

A similar method was used in computing for boars values of s for

TABLE 2. SELECTION DIFFERENTIALS IN THE MINNESOTA NO. 1 AND MINNESOTA NO. 2 FOR LITTER SIZE AT BIRTH AND NUMBER OF PIGS WEANED

Year	LITTER SIZE AT BIRTH						NUMBER PIGS WEANED					
	Minnesota No. 1			Minnesota No. 2			Minnesota No. 1			Minnesota No. 2		
	Males	Females	Av.	Males	Females	Av.	Males	Females	Av.	Males	Females	Av.
1938	.74	—.05	.34				.74	.37	.56			
1939	.32	.31	.32				.57	.50	.54			
1940	.77	.81	.79				.82	1.59	1.20			
1941	.98	1.29	1.14				1.33	1.68	1.50			
1942	.84	1.14	.99	—.09	.62	.26	1.69	1.29	1.50	—.04	.64	.30
1943	.56	1.02	.79	—.24	.30	.03	1.91	1.20	1.56	1.22	.76	.99
1944	.67	.59	.63	.37	.57	.47	—.24	.66	.21	.48	1.85	1.16
1945	.67	.70	.68	—.04	.59	.28	—.69	.22	—.24	1.01	1.35	1.18
1946	.40	.30	.35	.67	1.14	.90	—.28	.72	.22	1.38	1.45	1.42
1947	.45	1.50	.98	.89	.28	.58	.90	.88	.89	2.27	.71	1.49
1948	.67	1.40	1.04	.60	1.02	.81	.44	1.40	.92	1.36	1.49	1.42
Av.	.64	.82	.73	.31	.65	.48	.65	.96	.81	1.10	1.18	1.14

pigs weaned (table 2), the only difference in procedure being due to the fact that no correction for age was used on the records of the boar's dam. Instead the dam's one gilt record was subtracted from the gilt herd average of the year in which the dam made the record. Half of this difference furnished the estimate of the selection differential in the boar. The differential was weighted in the same manner as described for litter size at birth.

Results and Discussion

In the Minnesota No. 1, 351 different females and 50 different boars were selected to be sires and dams of the spring farrows of 1939 to 1949, inclusive. Some of these individuals were used in two or three different years. If they were counted as different individuals each spring, then there would appear to be 488 females and 74 males. In the Minnesota No. 2, 29 different boars and 166 different females were used during the years 1943 to 1949 inclusive. They would appear as 35 boars and 224 females if they were counted as different individuals each season.

Values of s computed in each line are given in table 2. The average selection differential for size of litter at birth for the Minnesota No. 1 was .73 pig per litter; for the No. 2 it was .48 pig per litter. When the two lines are compared in detail on the basis of the results in table 2, it is seen that the average selection differentials in the No. 2 are smaller for initial litter size, but larger for litter size at weaning. Apparently the larger differential for number of pigs weaned in the No. 2 was not because just as large differentials could not be attained in the No. 1; during the first six years in the No. 1 selection differentials averaged larger than for the first six years in the No. 2. In 1944 and in following years in the No. 1, there was much less emphasis on number of pigs weaned, but there was about the same amount of selection for number of pigs at birth.

The average values of s (table 2) used with those values of H, b, I, and G calculated from the data or chosen from the literature were used to determine the theoretical annual rates of change in table 3. The "actual" rates of change in the number of pigs farrowed and in the number of pigs weaned were determined by the regressions of the yearly means of number of pigs farrowed by gilts and number of pigs weaned by gilts (table 4) on time. (See also figures 1 and 2.) Comparison of actual rates and theoretical rates in the Minnesota No. 1 shows rather close agreement. In the No. 2 there is only qualitative agreement. A decline in litter size at birth was predicted for the No. 2, but the

TABLE 3. THEORETICAL AND ACTUAL ANNUAL RATES OF CHANGE IN LITTER SIZE AND NUMBER OF PIGS WEANED IN THE MINN. NO. 1 AND MINN. NO. 2

	Minn. No. 1	Minn. No. 2
Live Pigs Farrowed		
Theoretical rate due to:		
(1) Selection (sH/G)	.077	.057
(2) Inbreeding (bI)	—.118	—.115
(3) Selection and inbreeding (m)	—.041	—.058
Actual rate:	—.037	—.183
Number of Pigs Weaned		
Theoretical rate due to:		
(1) Selection (sH/G)	.125	.195
(2) Inbreeding (bI)	—.416	—.403
(3) Selection and inbreeding (m)	—.291	—.208
Actual rate:	—.260	—.155

predicted decline was not as great as that which apparently occurred. In the case of the number of pigs weaned, the actual rate of decline was not as great as expected. The direction of change in performance of both lines was in the predicted direction.

For the reason that there are a number of difficulties inherent in the methods used in computing the theoretical rates as well as the actual

TABLE 4. NUMBER OF PIGS FARROWED AND NUMBER OF PIGS WEANED BY GILTS IN THE MINNESOTA NO. 1 AND MINNESOTA NO. 2 INBRED LINES

Year	Minnesota No. 1			Minnesota No. 2		
	No. of gilts	Av. no. pigs fwd.	Av. no. pigs wnd.	No. of gilts	Av. no. pigs fwd.	Av. no. pigs wnd.
1938	19	9.63	7.63			
1939	39	8.82	7.05			
1940	38	9.18	7.03			
1941	32	8.03	6.34			
1942	28	7.79	6.11	13	6.23	5.54
1943	34	9.09	5.09	19	9.11	5.47
1944	20	9.60	4.50	15	7.93	3.67
1945	33	8.91	5.61	33	8.30	4.67
1946	27	9.59	6.15	26	8.08	5.69
1947	48	7.77	2.58	33	6.97	3.03
1948	31	8.42	6.19	19	6.11	4.84
1949	21	9.19	5.90	21	7.38	4.57

rates of change in litter size and in number of pigs weaned it would be surprising if the theoretical rates of change were in very close agreement with the actual rates. One of the difficulties is knowing exactly what values of H and b to use for each trait, since both statistics are subject to rather large errors of sampling.

Sources of error in estimating actual rates of change come from the practical limitations of the data, such as unequal numbers on which the annual line means are based. There may also be trends in performance

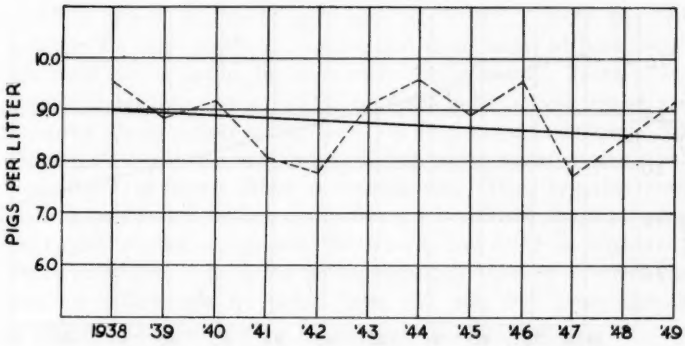

FIG. 1-A. ANNUAL AVERAGE NUMBER OF LIVE PIGS PER LITTER FARROWED BY MINNESOTA NO. 1 GILTS.

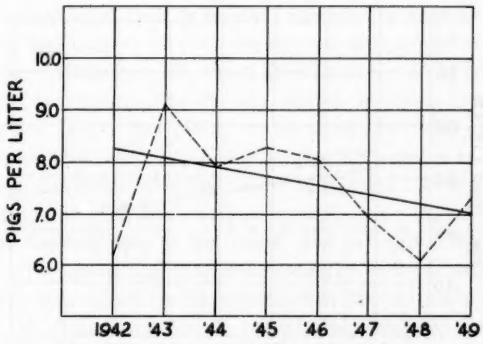

FIG. 1-B. ANNUAL AVERAGE NUMBER OF LIVE PIGS PER LITTER FARROWED BY MINNESOTA NO. 2 GILTS.

associated with environment and managerial practices. "Actual" rates of change, as computed in this study, may not therefore, reflect only genetic change. Close quantitative agreement between theoretical and actual rates of change in repeated studies would be required to support definite conclusions that selection is always as effective as theoretical considerations suggest. Because of the several possibilities for the introduction of error in the calculation of theoretical rates of change and because of the large annual variations which obscure the relatively

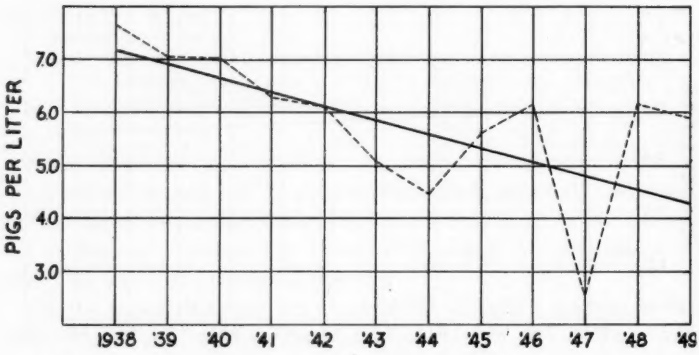

FIG. 2-A. ANNUAL AVERAGE NUMBER OF PIGS WEANED PER LITTER BY MINNESOTA NO. 1 GILTS.

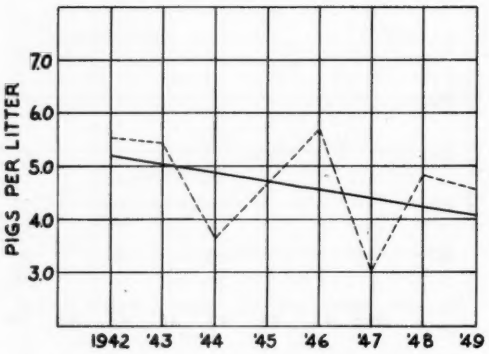

FIG. 2-B. ANNUAL AVERAGE NUMBER OF PIGS WEANED PER LITTER BY MINNESOTA NO. 2 GILTS.

much smaller changes in genetic merit, it is doubtful if it will ever be possible to design large animal experiments which will afford satisfactory empirical confirmation of theoretical calculations.

After allowing for the fact that there are several possible sources of error in both the actual and predicted rates, the results still seem to suggest that selection was effective in slowing down the expected decline from inbreeding in the number of pigs farrowed and the number of pigs weaned, especially when the two lines are compared. In the Minnesota No. 1 there was more selection for large initial litter size and there was a slower rate of decline in litter size. In the No. 2 there was more selection for large litters at weaning and there seems to have been a less rapid rate of decline in the number of pigs weaned. Taken at face value such results suggest that the emphasis in the re-selection of sows should be placed usually on the size of the litter at weaning, disregarding the size of the litter at farrowing. Young replacement females should come from the largest litters at weaning time. Thus, selection would tend to be directed toward the optimum initial litter size from which the largest number of pigs could be weaned. This could be important if the largest litters at birth are not the largest at weaning, in which case selection differentials for initial litter size may not mean also the largest selection differentials for number of pigs weaned.

Summary

The amount of selection as measured by the average deviation of selected parents from their generation averages in number of pigs farrowed alive and number of pigs weaned was determined in two inbred lines of swine (Minnesota No. 1 and Minnesota No. 2) in the Minnesota swine breeding project. The average annual selection differentials for the Minnesota No. 1 and No. 2, respectively, in number of pigs per litter farrowed alive was .73 pig and .48 pig; for number of pigs weaned per litter the selection differentials were .81 pig and 1.14 pig. Theoretical rates of change in each trait were computed, using the selection differentials, the heritabilities of the traits, and estimates of the effect of inbreeding. The theoretical rate was compared with the estimate of the "actual" rate determined by the regression of the trait on a time scale in years. In the Minnesota No. 1, actual and predicted rates were in rather close agreement; in the No. 2 there was only agreement as to direction of change. Selection appeared to accomplish most in the line where most selection was practiced.

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MECHANICAL MEASUREMENT OF FATNESS AND CARCASS VALUE ON LIVE HOGS[1]

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EFFORTS to improve carcass value in hogs by genetic means have been handicapped for lack of a rapid and accurate method of measuring carcass value in prospective breeding animals. Visual estimates and linear body measurements are not sufficiently accurate (Phillips, *et al.*, 1939, and Hetzer, *et al.*, 1950). They also have an additional disadvantage in that the most accurate external criteria of high carcass value are narrow, shallow middles and long bodies and legs. Direct carcass measurements which require slaughter of the individual can provide information for selection between sires or families, but these are likely to lengthen generation interval and are too laborious to apply upon a large scale.

The purpose here is to describe a simple and rapid "probing" method of measuring backfat thickness on live hogs. The method causes little discomfort to the pigs, and four measurements per pig can be taken in one minute. Correlations between the measurements of backfat on live hogs and carcass backfat measurements, percentage of primal cuts, and cross-sectional area of lean in the loin and in the ham are given to indicate the accuracy of the method as a criterion of carcass value.

Methods and Data

Measurements were taken on 96 pigs fed in Record of Performance trials at the Iowa Agricultural Experiment Station in cooperation with the Regional Swine Breeding Laboratory, during the fall and winter of 1950–51. The pigs were single crosses and three-breed crosses of inbred lines of Poland, Landrace, Duroc, and Chester White. Four pigs of each litter were fed as a group in a small concrete pen from weaning to a weight of about 215 pounds. Forty-eight litters were self-fed a ration of shelled corn and protein supplement free choice. The first and third pigs of each litter to reach a weight of 215 pounds were slaughtered. The pigs were taken off feed but had access to water 24 hours prior to slaughter.

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Live Animal Measurements

The pigs were restrained by looping one end of a small rope around the upper mandible and the other end around a post or other stationary object. Incisions about ¼ inch deep and ½ inch long were made with a scalpel or lancet through the skin. A narrow metal ruler with a blunt end was pressed through the soft fat to the firm tissue underneath. Sufficient resistance is encountered when the ruler reaches the juncture of the fat and firm tissue to indicate that the ruler has passed through the fat. Pressure on the ruler is relaxed for an instant and the reading marked at skin level with the thumb nail or a sliding metal clip on the ruler.

There is practically no danger of penetrating the well-defined muscles of the body which are covered with a definite layer of connective tissue, such as the longissimus dorsi or muscles of the shoulder or ham, particularly if the incisions in the skin are made transversely to the underlying muscle fibers. Penetration may occur between the longissimus dorsi and the scapula if incisions are made directly over the shoulder.

In preliminary trials, an injection of local anesthetic at the site of the proposed incision caused greater discomfort to the pigs than an incision made without anesthesia. Shaving the hair at the site of the incision and applying antiseptic proved to be unnecessary in preventing infection; no noticeable ill effects have occurred among the many animals retained under observation after they were measured. Hence the technique in use at the time the present data were collected was a relatively simple one involving four steps; (1) Restraining the pig; (2) making incisions at the chosen sites; (3) measuring the fat from the skin to the underlying firm tissue, and (4) recording the measurements.

The actual sites of the measurements in this study were chosen more or less arbitrarily after some preliminary experimentation. Three of the sites were about 1½ inches off the midline of the body above the longissimus dorsi. The first of these was immediately behind the shoulder; the second was the middle of the back, while the third was the middle of the loin. The fourth site was the middle of the loin over the exact midline of the body.

Carcass Measurements

The carcasses were split immediately after slaughter and were chilled for 72 hours at a temperature of approximately 34° F. Thickness of backfat was measured to the nearest one-tenth inch at the first, seventh

and last thoracic vertebrae, and at the last lumbar vertebra. The right half of each carcass was cut as described by Hankins and Hiner (1937). Maximum length and maximum width of the longissimus dorsi were measured from the trimmed loin cut at the last thoracic vertebra. Maximum length and maximum width of the lean area of the face of the untrimmed ham were measured. About ¼ inch of fat was left on both loin and ham after trimming. The belly was trimmed above the teat line and squared at the shoulder and flank ends. The shoulder was removed at the third thoracic vertebra and divided into the picnic ham

TABLE 1. MEANS AND STANDARD DEVIATIONS OF VARIABLES

Variable	Average	Standard deviation
Age at slaughter (days)	151.7	10.3
Shrunk live weight at slaughter (pounds)	211.3	5.3
Average of 4 carcass backfat measurements	1.61	0.14
Individual live hog backfat measurements		
Behind shoulder (inches)	1.73	0.23
Middle of back (inches)	1.38	0.19
Middle of loin over longissimus dorsi	1.26	0.18
Middle of loin over vertebra (inches)	1.37	0.17
Average of 4 live hog backfat measurements	1.43	0.17
Lean loin area (square inches)	6.34	0.89
Lean ham area (square inches)	19.02	1.39
Primal cuts (percent of shrunk live weight)	47.77	1.66

and Boston butt. Weights were taken on all cuts to the nearest one-tenth pound.

The particular carcass measurements studied were as follows: (A) average of the four backfat measurements; (B) loin area as measured by the product of maximum length and maximum width; (C) lean ham area as measured by the product of maximum width and maximum length, and (D) percentage primal cuts as measured by the combined weights of five cuts, loin, ham, bacon, picnic shoulder and Boston butt, divided by the shrunk live weight taken immediately before slaughter.

Results and Discussion

The pigs grew rapidly, reaching slaughter weight at slightly less than five months of age (table 1). The average depth of backfat on the carcasses was 1.61 inches at the four sites measured. Although this is near the accepted optimum for fatness, there was considerable variation between individuals, particularly with reference to breed-group differ-

ences. The computations were made on an intrabreed-group basis to eliminate breed differences in fatness and conformation. The primary effect of this was to decrease slightly the correlations between any two measures of fatness and to increase slightly those between measures of fatness and leanness. Seventeen of the 96 pigs were gilts, most of which were in two of the breed-groups. Hence the intrabreed-group analysis eliminated most of the sex difference.

Depth of fat was greater at the carcass locations than at the live-hog locations most nearly corresponding to them. The average of the four carcass measurements was 1.61 inches while the average of the four live-hog locations was 1.43 inches (table 1). The carcass measurements

TABLE 2. CORRELATIONS BETWEEN BACKFAT THICKNESS AND LOIN AREA, HAM AREA AND PRIMAL CUTS

Variable	Lean loin area	Lean ham area	Percentage primal cuts
Average of 4 carcass backfat measurements	—.414	—.427	—.450
Average of 4 live hog backfat measurements	—.442	—.538	—.499
Individual live hog backfat measurements			
Behind shoulder	—.483	—.468	—.468
Middle of back	—.321	—.455	—.391
Middle of loin over longissimus dorsi	—.412	—.560	—.446
Middle of loin over vertebra	—.332	—.462	—.449

were made from the skin to the top of the vertebra while three of the live-hog measurements were made from the skin to the top of the longissimus dorsi.

The correlations between the average backfat thickness on the carcass and the individual live-hog measurements at the four sites were as follows: Behind shoulder, .79; middle of back, .59; middle of loin over longissimus dorsi, .67; middle of loin over vertebra, .73; and average of the four live-hog measurements, .81. The location behind the shoulder was the most accurate single indicator of carcass fatness. That correlation is significantly greater than those involving the middle of the back and the middle of the loin over the longissimus dorsi. Because the average of the four carcass measurements is common to all of the correlations, the value $(r_{y1}-r_{y2})\sqrt{(n-3)/2(1-r_{12})\,(1-R^2)}$ which is distributed approximately as t, was used as suggested by Hotelling (1940) in making the tests of significance.

All of the correlations involving backfat measurements and the measures of carcass value are negative (table 2). Surprisingly, the

average of the live-hog measurements appears to be a more accurate indicator of leanness and carcass value than does the average of the carcass backfat measurements. The difference between the two correlations involving lean ham area (—.427 and —.538 in table 2) is statistically significant and the smaller differences between the correlations involving lean loin area and percentage primal cuts point in the same direction. The only logical reason for this seems to be that three of the live-hog measurements are made from the skin to the muscle instead of from the skin to the vertebra.

The middle of the back is definitely the poorer location for measuring backfat on live hogs. Correlations involving that location are lowest for the three measures of carcass value as well as for carcass backfat. The locations behind the shoulder and at the middle of the loin over the longissimus dorsi are most accurate if the measurements are to be used as indicators of carcass leanness. The former is significantly more accurate as a measure of lean loin area while the latter is significantly more accurate as a measure of lean ham area. This seems to be logical, since the latter location is much closer to the ham.

Considerably greater accuracy in measuring carcass value is possible than is indicated by the foregoing correlations. Chemical methods, as illustrated by the solubility of antipyrine in body water (Kraybill *et al.*, 1951), may have greater accuracy than the probing technique described herein. However, the latter has considerably greater usefulness in being more rapid and easier to apply as well as in the fact that the results are immediately available. The latter consideration is of considerable practical importance because the animals should be measured at approximately market weight so those not selected for breeding can be sent immediately to market. There is also considerable likelihood that greater accuracy can be attained by refining and extending the probing technique. For example, there seems to be no reason why the fat covering of the shoulder and ham cannot be measured as easily as that over the back.

As far as the results of this study go, the preferred locations are over the longissimus dorsi about 1½ inches off the midline of the body just behind the shoulder and at the middle of the loin. Since little additional labor is required in making additional measurements, both sides of the body could be sampled at these locations. In this event, each location should be chosen as independently as possible of the others.

Summary

A probing technique for measuring fat covering on live hogs is described. The method is very quick and easy to apply and causes little discomfort to the pigs. The correlation between the average of four backfat measurements taken on carcasses and on live hogs was .81. Measurements made on 96 live hogs were slightly more accurate as indicators of leanness and percentage primal cuts than were carcass measurements of backfat thickness. The most accurate locations were just behind the shoulder and at the middle of the loin about 1½ inches off the midline of the body.

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A METHOD FOR MEASURING THE PROPORTION OF FAT AND LEAN TISSUE IN SWINE CARCASSES

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SWINE carcass evaluation is an important problem which has received considerable attention in recent years. This is due to the lower price of lard as compared with the price of lean cuts of pork and the prices paid for live hogs.

Several studies on carcass measurements have reported strong relationships between average backfat thickness and fat content of the carcass (Hankins and Ellis, 1934; Warner, Ellis and Howe, 1934; Aunan and Winters, 1949; and Engelman *et al.*, 1950). Brown, Hillier and Whatley (1951) measured specific gravity of swine carcasses and found high correlations between this factor and percentage of lean cuts, percentage of fat cuts and backfat thickness.

Sampling techniques for beef and lamb carcass evaluation have been studied and developed by several workers (Hankins and Howe, 1946; Hankins, 1947; and Palsson, 1939). One of the objectives of this study was to investigate the possibility of using a coring device for carcass sampling to estimate the fat and lean content of the swine carcass.

Materials and Procedure

Data were collected on 70 swine carcasses which were from hogs of 11 different breeds and crosses.

The hogs were from groups that were raised under rather similar environmental conditions. All hogs were slaughtered "shipper style," split down the median line, hams unfaced and left in the cooler at 34° F. for a 48 hour chilling period.

The live weight of the hogs ranged from 170 to 327 pounds and the carcasses ranged from 122 to 254 pounds. The cold carcass weight was used instead of live weight of the animal for determining percentages.

After the normal cooling period, samples of fat and lean tissue were taken from the carcasses with a coring device. Figure 1 shows the coring device with its measurements. Figure 2 shows the locations at which the carcasses were probed with the coring device.

The following includes the description of the location of samples taken from the carcasses:

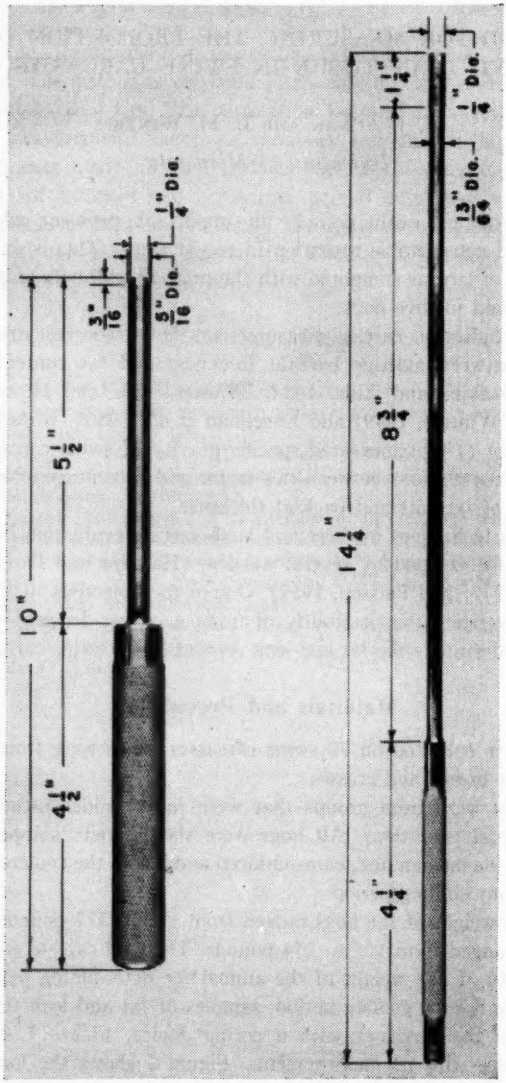

Figure 1. The carcass coring device with detailed measurements.

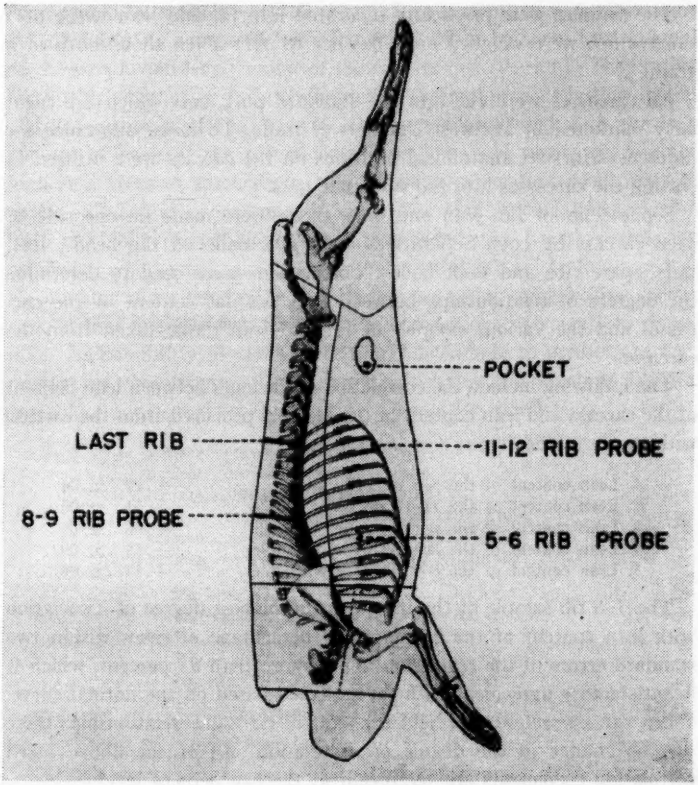

Figure 2. A diagram of a swine carcass with the location of the positions at which the carcasses were sampled with the coring device

1. 5–6 rib belly was taken between the fifth and sixth ribs at a point which is half the distance between the dorsal edge of the sternum and the ventral edge of the spinal column.
2. 8–9 rib of the loin was taken between the eighth and ninth ribs and one inch from the split side (median line) directly through the carcass.
3. 11–12 rib belly was taken from the centermost area of the space between the subcostal arches of the 11th and 12th rib cartilages.
4. Last rib of loin was taken an inch from the split side (median line) at the last rib directly through the carcass.
5. Pocket of belly was taken at a point which is half the horizontal distance between the ventral and dorsal edges, and one inch above the anterior edge in the pocket of the belly.

The samples were physically separated into fat and lean tissue portions which were weighed on a balance to over a ten thousandth of a gram.

All carcasses were cut into the standard pork cuts which are regularly marketed in Midwest channels of trade. To make allowance for differences in size, anatomical positions on the carcass were utilized in cutting the carcasses into the wholesale cuts.

Separations of fat, lean and bone tissue were made on one side of each carcass by cuts. Separations were not made on the heads, feet, tails, spare ribs and neck bones. Correlations were used to determine the degrees of relationships between lean and fat content of the carcasses and the various samples of fat and lean tissue taken from the carcasses.

The following include the correlation coefficients between lean content of the carcass and lean content of the samples procured from the carcass with the coring device:

1. Lean content of the 5–6 rib belly sample	+.79	±.04
2. Lean content of the 11–12 rib belly sample	+.71	±.06
3. Lean content of the pocket of belly sample	+.68	±.06
4. Lean content of the last rib of loin sample	+.54	±.09
5. Lean content of the 8–9 rib of loin sample	+.52	±.09

The 5–6 rib sample of the belly has the highest degree of association with lean content of the carcass. The percentage of cases within two standard errors of the regression line is more than 95 percent, which is in satisfactory agreement with the expected based on the normal curve. When carcass weight was held constant in the same relationship, there was no change in the degree of association. All of the above listed correlation coefficients are significant at the one percent level.

The five primal cuts (percentage) were correlated with percentage of lean tissue of the core samples. The following correlation coefficients and their respective standard errors were obtained:

1. 5–6 rib belly sample	+.61	±.08
2. 11–12 rib belly sample	+.61	±.08
3. Pocket sample	+.54	±.09
4. Last-rib loin sample	+.48	±.09
5. 8–9 rib loin sample	+.47	±.09

The above correlation coefficients are not as high as those obtained for the lean samples correlated with lean content of the carcass. The difference is due largely to the belly (fat cut) being included in the five primal cuts. An additional factor contributing to lack of agreement between correlation coefficients is noted for the relationship between

four primal cuts (belly excluded) and lean content of 5–6 rib sample (+.67±.06) as compared with the relationship between lean content of the sample and lean content of the carcass (+.79±.04). The cutting error which cannot be fully eliminated is the major contributing factor. The lean content of the 5–6 rib sample was correlated with the lean content of each of the primal cuts. The ham and picnic showed the highest degrees of association; the following correlation coefficients of +.69, +.65, +.61, +.54 and +.53 are for: Ham, picnic, belly, Boston butt and loin respectively. The loin yielded the lowest correlation coefficient which emphasizes further the "cutting error." In this case it is the amount of fat left on the loin that is the major complicating factor.

A further analysis was made of the correlation coefficients to determine the possibility of using more than one sample to predict the lean content of the carcass. The following includes the symbols for the variables represented in the multiple correlation coefficients:

a=lean content of carcass
b=lean content of 5–6 rib probe
c=lean content of 11–12 rib probe
d=lean content of the pocket of belly probe
e=lean content of the last rib of loin probe
f=lean content of the 8–9 rib of loin probe

Ra.bc=.83	Ra.ce=.73
Ra.bd=.83	Ra.cf=.72
Ra.be=.80	Ra.de=.77
Ra.bf=.80	Ra.df=.73
Ra.cd=.78	Ra.ef=.59

None of the above multiple correlation coefficients are sufficiently higher than the gross correlations to justify using more than one probe sample to predict lean content of the carcass. It is recognized that the use of an additional carcass sample would increase the time and effort that is involved in separating and weighing the lean and fat tissues of the samples. This must be kept at a minimum if the testing of a large number of carcasses is the objective.

Correlation coefficients between fat content of the samples and fat content of the carcass are not as high as those for lean content of the carcass. The following show the correlation coefficients for these relationships:

1. 11–12 rib belly sample	+.54	±.09
2. 5–6 belly sample	+.52	±.09
3. Pocket belly sample	+.45	±.10
4. Last rib loin sample	+.45	±.10
5. 8–9 rib loin sample	+.35	±.10

None of the above correlation coefficients are high enough to justify their use in predicting the amount of fat a carcass contains with a reasonable degree of accuracy. Furthermore, their use in multiple prediction equations for fat content is limited by the high degree of association between samples of the carcass.

Average backfat thickness obtained from the three measures along the backfat edge of the split carcass was not as high in its relationship to fat content or 5–6 rib probe. For the relationship between average backfat thickness and fat content of the carcass, a correlation coefficient of +.70 was obtained; on the same relationship with carcass weight held constant, the correlation coefficient increased to +.78. This correlation is as high as that obtained for lean content of the 5–6 rib probe and lean content of the carcass and higher than any of those obtained for fat content of the probes and fat content of the carcass.

The problem of predicting the relative amounts of fat and lean tissue the carcass contains with the coring device was investigated by combining the weights of lean and fatty tissue of the carcass into a ratio. This was also done for the samples so that both could be correlated. The degree of relationship between the ratios of fat to lean tissue of the 5–6 rib sample and the carcass was determined. The correlation coefficient for this relationship was found to be +.79 ±.04. The ratios of fat to lean in the 5–6 rib samples ranged from .28 to 1.36; for the carcasses, they ranged from .49 to 1.34. By the ratio is meant the proportion between fatty tissue and lean tissue of the carcass or sample, whichever the case may be. For every pound of fatty tissue, there are so many pounds and/or parts of a pound of lean tissue. The regression equation calculated for relationship of the ratios of fat to lean tissue between the 5–6 rib sample and the carcass is $x=.3855+.7187y$; x being the dependent variable, the ratio of fatty tissue to lean tissue of the carcass. Two standard errors of estimate about the regression line include more than 95 percent of the cases and one standard error of estimate about the line included more than 75 percent of the cases. In checking the bi-variate distribution, it was noted that heavy and light carcasses were found throughout the scatter.

Summary

Seventy hog carcasses were used in the collection of data to study the use of a sampling technique for estimating the proportion of fat and lean tissue of swine carcasses.

The results indicated that a coring device may be used to procure

samples of fat and lean tissue for purposes of estimating the relative amounts of fat and lean tissue a carcass contains.

Of the five positions on the carcass tested, the 5–6 rib sample offers the best index of the lean tissue of the carcass when percentage figures are used. This, however, does not hold true for percentage figures in estimating fat content.

There is considerable variation in the degrees of relationship between percentage of lean of the various samples. No additional improvement in correlation coefficients was obtained by combining several samples to use in multiple prediction equations.

A high degree of association exists between lean content of the samples and percentage of five primal cuts.

The ratio of fat to lean tissue in the 5–6 rib samples is highly associated with the ratio of fat to lean tissue of the carcass. This relationship has more merits for its use in carcass appraisal than any of the others tested in this study.

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ALLEVIATION OF COBALT TOXICITY IN CALVES BY METHIONINE ADMINISTRATION[1]

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IT is well recognized that cobalt administered orally is the most effective means of correcting the syndrome of cobalt deficiency. The work of Ray *et al.* (1948) and Keener *et al.* (1950) has shown that cobalt administered intravenously was much less effective in relieving cobalt deficiency in sheep than that given orally. The fact that some response was secured with intravenously administered cobalt makes it important to consider this fact when studying the physiological role of cobalt in the ruminant.

While conducting studies on the role of cobalt in the ruminant, it was noted by the authors that high dosages of cobalt administered intravenously to dairy calves produced toxicity symptoms of varying severity depending upon the size of dose given. The oral, subcutaneous and intraperitoneal administration of high dosages of cobalt produced symptoms of toxicity in the rat as was reported by Waltner and Waltner (1929), Le Goff (1928) and Griffith *et al.* (1942). Large oral doses of cobalt have also been shown to be toxic and cause depressed appetite and body weight losses in dairy calves (Ely *et al.*, 1946, 1948; Keener *et al.* 1947, 1949) and sheep (Becker and Smith, 1951). It should be emphasized that the dosage level of cobalt necessary to produce toxicity symptoms in the ruminant is many times the daily requirement or the recommended therapeutic dosage level. There is considerable evidence that the occurrence of cobalt toxicity in ruminants under practical conditions is extremely unlikely. Geyer, Rupel and Hart (1945) fed 50 mg. of cobalt per day to three Holstein heifers weighing about 500 pounds each for 8 weeks without producing polycythemia. This dosage was calculated to be at least ten times the recommended therapeutic dosage. Keener *et al.* (1947, 1949) found that at least 50 mg. of cobalt per 100 pounds of body weight per day to growing dairy calves was necessary to produce detrimental effects and pointed out that this was 100 times the amount of cobalt normally consumed from

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commercial cobalt supplemented dairy rations. Ely *et al.* (1946, 1948) reported that it was necessary to feed above 40 mg. of cobalt daily per 100 pounds of body weight to produce toxic effects in dairy calves which is comparable to the dosage level reported by Keener *et al.* (1947, 1949). Josland (1937) did not get uniform results with sheep when he drenched four healthy ewes with 5 mg. of cobalt per kilogram of body weight and found that one developed polycythemia, one remained normal and two showed mild anemia. Becker and Smith (1951) recently found that fattening yearling sheep were tolerant of a daily dosage of 160 mg. of cobalt per 100 pounds of body weight for a period of eight weeks without any apparent undesirable effects. This intake is nearly 1600 times the estimated requirement of these sheep. Dosages of 200 mg. or more daily per 100 pounds of body weight were necessary to produce a depraved appetite and body weight losses. These results emphasize the wide margin of safety that is present between the recommended therapeutic dosages of cobalt to ruminants and the dosage levels necessary for the production of toxicity symptoms.

Several investigators (Burk *et al.* 1946, Kondo and Hayashi, 1928, Michaelis, 1929, Michaelis and Yamaguchi, 1929, Schubert, 1931, and Schubert, 1933) reported that cobalt forms chelate ring compounds with certain amino acids. The detoxification of heavy metals by cysteine, cystine and methionine was reported by Michaelis (1929), Michaelis and Yamaguchi (1929), Vickery and Leavenworth (1930) and Schmidt (1945).

The object of this investigation was to determine the effect of the intravenous administration of cobalt in dairy calves and to study the effect of previously injected methionine on the alleviation or prevention of the symptoms of cobalt toxicity.

Experimental Procedure

Eighteen Holstein and Jersey bull calves ranging from one week to six months of age were used in these studies. They were fed a ration of whole milk at the rate of one pound per 10 pounds of body weight, a 20 percent protein grain mixture consisting of 33 parts yellow corn, 33 parts oats, 20 parts dried skim milk, 13 parts linseed oil meal and one part salt, and alfalfa hay *ad libitum*. All animals were in good health when started on the experiment.

Cobalt was injected into the jugular vein as an aqueous solution of cobalt sulfate. The concentration of elemental cobalt in the solution varied so that not more than 10 ml. of solution was injected into any

animal. Seven calves were injected with an aqueous solution of methionine three to five minutes previous to the injection of the cobalt sulfate in order to study the effect of methionine on the toxicity of intravenously administered cobalt. The doses of methionine were 500 mg. for three calves and 1000 mg. for four calves.

Results and Discussion

When the 10 calves were injected with from .37 to .82 mg. of elemental cobalt per pound of body weight, toxic symptoms were observed in all cases. The toxic symptoms of intravenously administered cobalt were lacrimation, salivation, dyspnea, incoordination, defecation and urination.

Lacrimation and salivation were very pronounced during the early stages of the attack and the animals breathed very rapidly and with apparent pain and discomfort. Muscular incoordination was evident soon after the injection and the animals were unwilling or unable to stand for varying lengths of time depending upon the size of dose and the severity of the attack. The animals showed signs of discomfort and lassitude while lying down. Urination and defecation were most pronounced while the animals were lying down. The results with dairy calves are in agreement with those resulting from administration of cobalt salts in man, dogs, and rabbits as reported by Le Goff (1928), which indicated a specific stimulation of the thoracico-lumbar sympathetic system.

In most of the cases of toxicity the animals were standing and appeared normal about two hours after injection. Some animals which received smaller doses recovered in a shorter period of time.

The tolerance of dairy calves for intravenously administered cobalt without showing toxicity symptoms was not established. Animal C600, however, tolerated 0.14 mg. of cobalt per pound of body weight without showing symptoms of toxicity and all other animals which received more than 0.37 mg. per pound of body weight showed the symptoms described. Animal C623 was injected with 4 mg. of cobalt per pound of body weight and recovered. The animal was unable to stand until eight hours after the injection.

Several animals received weekly injections of cobalt and did not appear to build up tolerance or sensitivity to the cobalt with repeated injections. All of the animals apparently recovered completely within 12 hours.

Ten animals received intravenous doses of cobalt ranging from 40 to 100 mg. which corresponded to from .37 to .82 mg. of cobalt per pound of body weight. In addition, seven animals received intravenous doses of cobalt ranging from .49 to .84 mg. of cobalt per pound of body weight and also received previous intravenous administration of either 500 or 1000 mg. of methionine per animal. The weight and age of the calves, the amount of cobalt and methionine injected and observations on the occurrence of toxic symptoms are presented in table 1. When methionine was administered previous to the injection of cobalt there

TABLE 1. THE EFFECT OF METHIONINE ON THE TOXICITY OF COBALT TO THE CALF

Calf	Age	Weight	Methionine[1]		Cobalt[2]		Remarks
	(day)	(lb.)	(mg.)	(mg./lb.)	(mg.)	(mg./lb.)	
C600	176	295			40	.14	No effects
C603	119	108			40	.37	Toxic symptoms
C622	95	164			75	.46	Toxic symptoms
C605	185	160			75	.47	Toxic symptoms
C627	62	151			75	.50	Toxic symptoms
C605	62	75			40	.53	Toxic symptoms
C625	13	89			50	.56	Toxic symptoms
C626	57	134			75	.56	Toxic symptoms
C618	71	126			75	.60	Toxic symptoms
C607	151	114			75	.66	Toxic symptoms
C623	97	122			100	.82	Toxic symptoms
C618	74	126	500	4.0	75	.60	Very slight symptoms
C607	148	114	500	4.4	75	.66	No effects
C627	6	98	500	5.1	50	.51	No effects
C624	73	154	1000	6.5	75	.49	No effects
C625	68	146	1000	6.8	75	.51	Very slight symptoms
C628	70	120	1000	8.3	75	.62	No effects
C630	47	89	1000	11.2	75	.84	Very slight symptoms

[1] Mgs. of methionine in aqueous solution injected intravenously.
[2] Mgs. of elemental cobalt in an aqueous solution of cobalt sulphate injected intravenously.

was complete prevention or a marked decrease in the toxic symptoms manifested in all the animals studied. Three of the animals showed very slight symptoms of cobalt toxicity as was indicated by a small amount of urine leakage and lacrimation. None of these animals showed incoordination.

Griffith *et al.* (1942) have shown that the sulphur containing amino acids prevented cobalt toxicity in the rat when the cobalt and the sulphur containing amino acids were administered simultaneously either orally or intraperitoneally. Cobalt and methionine apparently form a coordination complex in the blood stream which is less toxic than cobalt *per se*, as evidenced by the lack of stimulation of the thoracico-lumbar sympathetic system as indicated by failure of occurrence of defecation,

urination, lacrimation, salivation, and muscular contraction. The failure of small amounts of intravenously administered cobalt to give the toxicity symptoms may be due to the presence of certain amino acids or other detoxifying substances naturally present in the blood stream combining with the small amount of cobalt.

Summary

Calves were able to tolerate intravenous injections of small doses of cobalt without symptoms of toxicity.

Larger doses of cobalt sulfate (0.3 mg. of cobalt per pound of body weight or more) caused symptoms of toxicity which were lacrimation, salivation, dyspnea, incoordination, defecation and urination.

The injection of methionine previous to the injection of cobalt prevented or markedly reduced the severity of these toxicity symptoms.

The lethal dose of cobalt injected intravenously is more than 4 mg. per pound of body weight for the dairy calf.

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A STUDY OF VITAMIN B_{12} DEFICIENCY IN THE CALF[1]

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IT is established that the ruminant requires a dietary source of cobalt and that on a ration deficient in this nutrient an anemia develops which is remediable by cobalt *per os* but is not remediable by cobalt injection (Marston and Lee, 1949). On the other hand, a cobalt deficiency has not been demonstrated in the monogastric animal. Gall and Loosli (1949) reported a decreased bacterial count and an alteration in the composition of the rumen flora in cobalt-deficient sheep, and Hale *et al.* (1950) demonstrated that oral cobalt administration increased the rumen synthesis of vitamin B_{12}. However, the apparent conclusion that cobalt functions in the ruminant only as a precursor for the synthesis of vitamin B_{12} is in conflict with the findings of Marston (1949) who found that the anemia produced in sheep fed low-cobalt rations does not respond to parenterally administered liver extract or pure antipernicious anemia factor. Becker, Smith, and Loosli (1949) also found that cobalt-deficient lambs did not respond to oral or intramuscular administration of vitamin B_{12}. In later publications Becker and Smith reported that, while vitamin B_{12} or folic acid injection did not influence the anemia, subcutaneous (but not oral) liver extract administration was curative (1949, 1951). Hale *et al.* (1950) ascribed no beneficial effect to vitamin B_{12} but reported that oral administration of a complete B-vitamin supplement reversed the anemia, indicating that cobalt may be important in the rumen synthesis

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of B vitamins other than vitamin B_{12}. Finally, Smith *et al.* (1951) obtained remission of cobalt deficiency in lambs by vitamin B_{12} injection and attributed their previous failure to do so to the use of inadequate quantities of the vitamin.

In view of the confusion surrounding the requirements of the ruminant for vitamin B_{12} and cobalt, an attempt has been made to produce an uncomplicated vitamin B_{12} deficiency in the calf.

Experimental

A series of purebred dairy calves representing several breeds was maintained on a "synthetic milk" diet in which "alpha protein" (an

TABLE 1. COMPOSITION OF "SYNTHETIC MILK" DIET

Ingredient	Amount	Vitamins added per liter			
	%		mg.		
Alpha-protein	29.4	Thiamine	.65	Folic acid	0.26 mg.
DL-methionine	0.6	Riboflavin	1.30	α-tocopheryl acetate	1.00 mg.
Cerelose	31.7	Pyridoxine	.65	2-Methyl-1, 4-naphthoquinone	0.26 mg.
Lard	30.0	Ca pantothenate	1.95	Vitamin A	2,000 I.U.
Mineral salts[1]	8.3	Nicotinic acid	2.60	Vitamin D-2	200 I.U.
		Choline chloride	26.0		
		Biotin	0.02		

[1] Identical to salts used by Johnson, James and Krider (1948) except that $CaCO_3$ was replaced with an equimolar quantity of Ca lactate.

isolated soy-protein obtained from the Glidden Co., Chicago) supplemented with methionine was employed as a source of protein. The composition of this diet (table 1) was similar to that used by Neumann, Johnson, and Thiersch (1950) in this laboratory to induce a vitamin B_{12} deficiency in the baby pig. Cobalt chloride was included in the salt mixture (Johnson *et al.*, 1948). The "alpha-protein," DL-methionine, and salts and cerelose were dissolved in hot water with a small amount of sodium bicarbonate, the lard and fat-soluble vitamins were incorporated by homogenization, and the mixture was pasteurized. Water-soluble vitamins were added in 30 percent alcoholic solution at feeding time, along with sulfathalidine which, when used, was employed at a rate of 3 gm. per liter of diet.

The calves were placed on experiment at one to three days of age and were fed *ad libitum*. All animals received a dose of 100,000 I.U. vitamin A and 10,000 I.U. vitamin D at the start of the experiment.

Results and Discussion

An early problem arose in preventing the animals from ingesting loose hair licked from the haircoat which formed hairballs in the rumen and threatened pyloric obstruction. This difficulty was obviated by the provision of wire mesh muzzles trimmed with leather which prevented

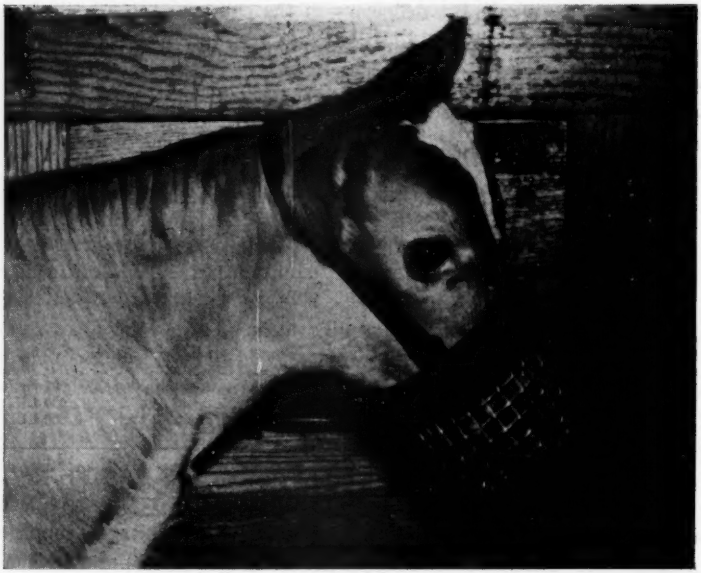

Figure 1. Photograph of calf showing muzzle used to prevent licking of haircoat.

the calves from licking their haircoats but permitted them to drink without interference from open pails (figure 1).

The growth data are presented graphically in figure 2. Calf 1 showed a growth plateau from the third week of the experiment to the twelfth, at which time he was treated with 1 ml. Lederle 15-unit liver extract (estimated to contain 20 μg vitamin B_{12} per ml. by biological assay) given intramuscularly at intervals of three days. As shown in figure 2, a resumption of growth occurred during the following week and for three weeks thereafter. It was accompanied by an average increase in food consumption of about one-third. No abnormal relationship was found in the differential blood cell series or in the total count, and

reticulocytes were absent from the blood both before and after therapy.

Calf 2 was maintained on the deficient diet without therapy for 16 weeks. Poor growth ensued after the seventh week, and during the final two weeks the animal became incoordinated and was unable to stand. Examination of the bone marrow revealed a somewhat low myeloid count as compared to that in the bone marrow of two control calves fed a natural farm diet. As reported earlier for the pig (1950), no immature megaloblast cells, such as occur in pernicious anemia, were found in these animals. A summary of representative marrow data as obtained from several calves is given in table 2. A trend to a

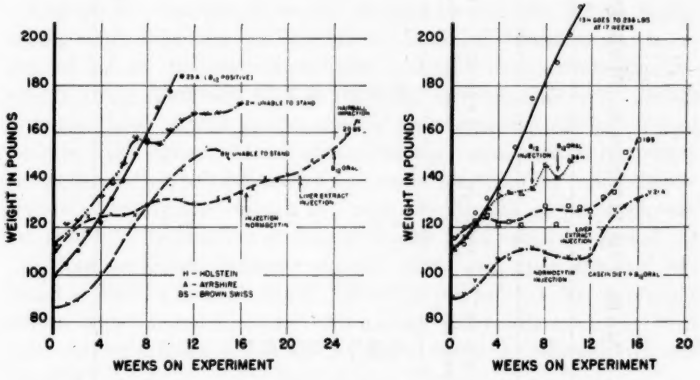

Figure 2. Growth curves of B_{12}-deficient and control calves.

depressed proportion of myeloid cells in the marrow of calves maintained on the basal diet is discernible.

Histopathological examination of the brachial and sciatic nerves of calf 2 showed an extensive demyelination in the Mahon preparations, accompanied by an increase in Schwann cells. Under Azan staining the neurokeratin network appeared to have been replaced by numerous orange-red staining particles. Sections of the spinal cord, cerebrum and cerebellum revealed no significant changes. The rumen was found to contain several hairballs, but there was no evidence of impaction either from post-mortem examination or from the appetite of the animal prior to autopsy.

Calf 8 exhibited fair growth on the basal diet for a period of 14 weeks, at the end of which time he displayed a lack of coordination and was unable to assume a standing position. Bone marrow inspection

TABLE 2. BONE MARROW VALUES FOR CALVES FED VITAMIN B_{12} DEFICIENT AND CONTROL DIETS

Calf No.	1	2	8	13	20		2a
Dietary history	4 wks. on basal plus liver extract	8 wks. on basal diet	8 wks. on basal diet	17 wks. on basal diet	3 days on cows' milk	16 wks. on basal diet	Practical farm ration
Percent myeloid cells	70	27	13	17	64	28	56
Percent erythroid cells	26	64	82	16	26	35	37
Percent lymphoid cells	4	9	5	67	10	37	7
Differential of myeloid series:							
Mature neutrophils	60	26	31	35	50	74	18
Metamyelocytes	17	44	31	43	20	15	36
Myelocytes	20	26	31	16	25	3	36
Myeloblasts	1	4	7		2		2
Eosinophils and basophils	2			6	3	8	8
Differential of erythroid series:							
Normoblasts, orthochromic	98	93	87	76	92	81	90
Normoblasts, basophilic	2	7	10	24	4	19	8
Normoblasts, macrocytic			3		4		2
Erythroblasts					present		

showed a low total myeloid count, as for calf 2, and an absence of large immature members of the erythroid series (table 2). Autopsy revealed a subnormal concentration of myelin around the peripheral nerves and several hairballs in the rumen, though no obstruction of the pylorus is believed to have occurred.

Despite continued maintenance on the alpha-protein diet for 17 weeks, calf 13 grew normally and showed no outward sign of deficiency at the end of this time. The composition of the peripheral blood was within the normal range, though the myeloid group in the bone marrow was significantly lower than that of vitamin B_{12}-treated or control calves fed a practical ration.

Calf 20 was maintained on the unsupplemented diet for 16 weeks, during which time very poor growth was obtained. Intramuscular injection of 0.5 ml. Lederle's normocytin (calculated to provide approximately 10 μg of vitamin B_{12}) at three-day intervals elicited no growth response. Subsequently, as shown in figure 2, intramuscular injection of Lederle liver extract (1 ml. twice weekly) produced a temporary resumption of growth followed by a second plateau. The oral administration of crystalline vitamin B_{12} at a rate of 100 μg per week was followed by a resumption of normal growth for a two-week period. At this time the animal suddenly stopped eating and, upon autopsy, was found to have a hairball firmly lodged in the pylorus. No significant lesions were observed during post-mortem examination. As in most other calves, examination of the peripheral blood for reticulocytes was negative.

Similar results were obtained with Calf 21. Following an initial period, growth in this calf diminished and stopped. Intramuscular injection of normocytin produced no growth response. In the twelfth week "Labco" casein was substituted for alpha-protein plus methionine in the diet, and, in addition, vitamin B_{12} (cyano-cobalamin) was administered orally at 100 μg per week. A marked growth response, as illustrated in figure 2, was obtained during the following five weeks. A slight reticulocytosis in the peripheral blood (0.1 percent of total red cells) was detected following the beginning of oral therapy in this calf, the only animal in which reticulocytes were found in the blood.

Calf 25 was maintained on the alpha-protein diet with crystalline vitamin B_{12} supplementation given intramuscularly at a rate of 0.6 μg per kilogram body weight per day. Over a period of 11 weeks this animal exhibited normal growth for the Ayrshire breed (1.14 pound per day) and showed no external evidence of a deficiency.

Calf 36, after maintaining a growth plateau for three weeks and then being subjected to vitamin B_{12} injection, relapsed after a one-week growth response. Subsequent oral therapy induced another short renewal of growth, following which the calf succumbed to bronchopneumonia. On the day following intramuscular therapy a marked reticulocytosis in the bone marrow was observed (23 percent of the marrow cells), and following oral administration of vitamin B_{12} 2 percent reticulocytes were detected in the marrow. Concomitant examination of the blood failed to reveal the presence of these cells.

Apart from the data obtained on the calves already listed, vitamin B_{12} treatment of several others, either intramuscular or oral, failed to elicit any growth response. Post-mortem examination of four of these animals revealed abnormalities of the gall bladder. In one, this organ was distended to a size greater than that of the liver, and no communication could be detected between the gall bladder and the intestine. Another calf showed evidence of obstructive jaundice as judged by post-mortem examination together with positive tests for the presence of bile acids and bile salts in the urine. While the presence of obstructive jaundice was not specifically observed in any other animals, such a condition was suspected in at least two additional calves, as evidenced by the light gray texture of the feces indicating an absence of bile pigments.

In several previous experiments in this laboratory which involved feeding calves a "synthetic milk" diet in which casein served as the protein source, no evidence of jaundice was found, and no difficulty was encountered in maintaining rapid growth. The difference in the ability of these two diets to consistently sustain growth in calves, therefore, appears to be associated with the source of protein. The only noticeable differences between the two diets were in apparent palatability and in the tendency to produce diarrhea, both factors favoring the casein "milk". Since the calves showed no disinclination to consume the alpha-protein diet in adequate amounts during the early part of the experimental period, its less appetizing flavor, as judged by human tastes, does not appear to have been a factor in its failure to promote continued well-being in some of the calves. While there was a considerable incidence of diarrhea in this experiment, no indication was found of damage to the intestinal epithelium. In occasional instances when scours became severe, streptomycin was administered orally, and relief from the condition was obtained consistently within 24 hours. The alpha-protein diet employed was identical with that which, when

supplemented with vitamin B_{12}, produced excellent growth in baby pigs over a period of eight weeks (1950).

Apart from the two calves in which jaundice was identified, a total of eight animals failed to respond to vitamin B_{12}, by either the intramuscular or oral route, after having exhibited a growth plateau. Three of these died of pneumonia after several weeks of growth stagnation. The remainder were eventually removed from the experiment and submitted to histopathological examination, which revealed no relevant information as to the cause of growth failure.

Urine analyses carried out for vitamin B_{12} activity using *Lactobacillus leichmannii* revealed a greatly reduced excretion on the part of the animals fed the basal diet. Thus, calves 36 and 35 excreted 0.02 and 0.01 mμg per ml. urine, respectively, after being maintained on the basal diet for six weeks, while calves 25 and 34, given vitamin B_{12} intramuscularly at 0.6 μg per kilogram body weight per day in addition to the basal diet, excreted typical levels of 3.6 and 2.1 mμg per ml. urine, respectively.

In contrast to the marked reticulocytosis observed in the vitamin B_{12}-deficient pig following therapy (1950), reticulocytes were detected in only one calf in this experiment. Moreover, no reticulocytes were found in normal calves, though Seyfarth (1927) has reported 0.1–0.2 percent in the blood of cattle. Their absence from the peripheral blood prompted an inspection of the bone marrow of later calves, and a stimulation of reticulocyte formation in this tissue was observed.

Summary and Conclusions

A series of calves maintained on a "synthetic milk" diet in which the protein source was alpha-protein from soybeans, supplemented with methionine, lard, cerelose, minerals, and vitamins other than vitamin B_{12}, exhibited a cessation of growth, poor appetite and, in some cases, incoordination. Examination of the peripheral nerves of some of these calves revealed varying degrees of demyelination, and the distribution of bone marrow cells showed a low proportion of the myeloid series.

In some animals growth was resumed following liver extract or crystalline vitamin B_{12} therapy, and a reticulocytosis was observed in the bone marrow. Other animals exhibited only transient responses to vitamin B_{12} therapy, intramuscular or oral, and still others failed to show any response at all. Biliary obstruction was observed in four calves and was suspected in at least two others. The fact that good growth was previously induced in calves fed a diet differing only in

that casein replaced alpha-protein plus methionine indicates that casein may have been supplying one or more factors in addition to vitamin B_{12} which are required for the normal development of the calf.

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FEED SUPPLEMENT CONTAINING AUREOMYCIN AND VITAMIN B_{12} FOR DAIRY CALVES[1, 2]

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THE value of vitamin B_{12} in promoting growth and increasing feed efficiency with swine has been shown by Hale and Lyman (1949), Cunha *et al.* (1950), and Catron and Wallace (1949). Bowland *et al.* (1950) found much greater gain with swine from an animal protein supplement containing aureomycin over an animal protein supplement with no residual aureomycin. Others (Catron *et al.* 1950, Cunha *et al.* 1950, Cuff *et al.* 1950) have noted increased gains, lower incidence of scouring and more thrifty appearance as a result of antibiotic feeding to swine.

Johnson (1950) in a preliminary report stated that the calf requires vitamin B_{12}. Both Rusoff and Haq (1951) and Hibbs and Pounden (1951) secured no growth increase from the supplementation of an all plant protein calf starter with a vitamin B_{12} concentrate. Milk was fed for 28 days and seven to nine weeks respectively. Collins, Dietrich and Elvehjem (1950) have reported that cow's milk contains 2–3 μg of vitamin B_{12} per liter. Williams and Knodt (1951) secured no greater growth when animal protein factor supplements were added to milk replacements for calves. Feeding an animal protein factor concentrate containing aureomycin, Bartley *et al.* (1950) concluded that growth of calves was enhanced by the prevention of scours.

The following experiment was conducted to study the influence of antibiotic feed supplement containing aureomycin on (1) growth and feed utilization efficiency and (2) incidence of scours with dairy calves.

Experimental

Fifteen heifer calves, 11 Holstein, three Ayrshire and one Guernsey, were placed on experiment as they were dropped in the experiment station herd. Every third calf was used as control. The length of the trial was 12 weeks.

[1] The antibiotic feed supplement was generously supplied by Lederle Laboratories. Pearl River, N. Y.

[2] Journal Series Paper No. 23; Vermont Agricultural Experiment Station.

TABLE 1. CALF FEEDING SCHEDULE

Age of Calf	Holstein		Ayrshire and Guernsey	
	whole milk	skim milk	whole milk	skim milk
	pounds		pounds	
1st day	with dam		with dam	
2–7 days	7		5	
2ndweek	9		7	
3	10		8	
4	10		8	
5	7	3	2	
6	3	7	2	6
7		12		10
8		12		10
9		10		8
10		8		6
11		6		4
12		4		4

The feeding schedule (table 1) was regularly followed in the herd. Each calf had access to good quality alfalfa hay *"ad libitum"* from birth. A 20 percent commercial calf starter (table 2) was fed at the rate of 1 pound per 50 pounds body weight up to a maximum of 4 pounds daily.

A vitamin B_{12} and antibiotic feed supplement containing not less than 1.8 milligrams of vitamin B_{12} activity per pound and not less than 1.8 grams of aureomycin hydrochloride per pound was added to the milk of the ten calves in the supplemented group. This was given as a

TABLE 2. COMPOSITION OF THE CALF STARTER

Ingredient	Lbs.
Alfalfa Meal	140
Yellow Cracked Corn	664.6
Corn Distillers' Dried Solubles	60
Fish Meal	60
Linseed Oil Meal	100
Molasses	160
Rolled Oats	300
Soybean Oil Meal	470
Irradiated Yeast (Brewers' type)	.4
Calcium Carbonate	5
Dicalcium Phosphate	30
Iodized Salt	10
Cobalt Sulphate—2 grams per ton	

finely ground powder at the rate of 9 grams per 100 pounds body weight. Weekly adjustments were made for gain in weight.

The calves were housed in individual pens equipped with hay rack and concentrate box for the initial part of the trial. Milk was fed from a nipple bottle or nipple pail. Later the calves were tied by a short chain and milk fed from an open pail. They were kept throughout the trial in a well lighted and well ventilated barn with no artificial heat. Wood shavings were used as bedding.

The calves were weighed on two consecutive days at weekly intervals, with the average of the two weights being recorded. Daily observations were made of the feces of all calves.

TABLE 3. AVERAGE GAIN IN WEIGHT AND FEED UTILIZATION EFFICIENCY OF CALVES AT 12 WEEKS OF AGE

Group	Number of Animals	Average Gain in Weight		
		Per calf (lbs.)	over normal (lbs.)	Per lb. of T.D.N.
Control	5	117.2	21.0	.59
Supplemented	10	136.0	38.1*	.64^{ns}

* mean difference significant
^{ns} mean difference not significant

Results and Discussion

The data concerning gain in weight and feed utilization efficiency are shown in table 3. The gain in weight is compared with the Ragsdale (1934) standard, which is commonly regarded as normal.

Gains in weight for all calves in both the control group and in the group receiving feed supplement containing aureomycin and vitamin B_{12} were above normal. However, the average gain for the supplemented calves was found on statistical analysis to be significantly greater than the average gain of the control calves. The daily rate of gain for the eight supplemented Holstein calves ranged between 1.48 to 1.83 pounds or an average of 1.68 pounds for the 12 weeks. For the Holstein controls the range was 1.42 to 1.57 pounds with an average daily gain of 1.50 pounds. The average daily gain for the Ayrshire calves was 1.42 pounds for the supplemented calf and 1.24 pounds for the control calves.

Although the gain per pound of T.D.N. consumed was higher for

the supplemented group than the control group, the mean difference was not significant. There was no evidence of increased calf starter consumption by the calves in the supplemented group. Hay consumption in both groups was small.

The condition of all calves throughout the experiment was good. There was no difference discernible in thriftiness, condition of the coat or general well-being of the two groups.

While observation of the feces of all calves was made daily, no case of scours was observed. The supplement had a growth-promoting effect even in the absence of scours. This is in contrast to the results of some other workers where scours were a factor influencing growth. All calves were kept in a well lighted and ventilated barn and carefully fed as to regularity and sanitary conditions. It appears that good management is still the most important factor in the prevention of scours in calves.

Summary

The feeding of a supplement containing aureomycin and vitamin B_{12} to calves receiving a liberal ration of milk, calf starter and good quality hay increased growth above that of the controls. No significant difference in utilization of feed nutrients was observed between the two groups. There was no evidence of scours in any of the calves throughout the duration of the trial. Growth of the supplemented calves was enhanced above that of the control animals even though scours, a conflicting factor in some other investigations, was absent.

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THE CAUSES OF BOVINE METESTROUS BLEEDING

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MOST of the work upon cyclical uterine bleeding has centered upon attempts to find the cause of menstruation in primates. Periodic bleeding in other species has received scant attention but a paper by Hartman (1931) suggests that it is much more frequent than is generally believed. In mammals it has been recorded in primates, dog, cow and guinea pig (Asdell (1946)) in the absence of pregnancy. In the cow it is not of universal occurrence. Krupski (1917) found it in 75 percent of heifers and in 48 percent of cows, while Trimberger (1941), also, observed it in about 90 percent of heifers and in 61 percent of cows. The writers have observed 109 cycles of 14 cows over three years old. Bleeding occurred in 46.8 percent. In heifers between 15 and 24 months old, 27 cycles of eight heifers showed an incidence of 92.6 percent. In mature cows the incidence varies; some cows bleed in all cycles, others in none, with most intermediate. These facts make it all the more difficult to determine the cause of this bleeding.

The problem is complicated further by the fact that periodic bleeding does not occur at the same time in the cycle in all species. In primates it is associated with the decline of the corpus luteum, with another period of bleeding in some animals at about the time of ovulation. In dogs it occurs during proestrum, while the graafian follicles are growing, but a second period, at the end of pseudopregnancy, when the corpus luteum has declined, is sometimes seen. In the cow it occurs just after ovulation; the heat period has terminated about 48 hours earlier. The cow, also, frequently shows microscopic bleeding at about ten days after heat. This has been found by Frei and Metzger (1926), Cole (1930) and Cupps (1945). In the guinea pig bleeding, usually microscopic in extent, may occur in the early metestrous phase.

In regard to the causes of the bleeding in primates, the work has been reviewed by Corner (1945). It appears to be due to withdrawal of a growth stimulus to the endometrium. This results in extensive degeneration and hemorrhage. It can occur in the ovariectomized monkey when injections of estrogen or of progesterone cease. Progesterone prevents estrogen deprivation bleeding and inhibits bleeding in the intact monkey. The prevailing opinion is that normal menstruation

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represents a progesterone withdrawal effect and that the intermenstrual bleeding sometimes encountered is due to estrogen withdrawal.

In the dog the hemorrhage is not associated with endometrial damage as it is in primates. The red blood cells are extravasated and appear in the lumen by a process of diapedesis, together with some epithelial denudation (Marshall and Jolly (1905)), (Evans and Cole (1931)). In their experimental work Meyer and Saiki (1931) found that bleeding occurs while estrogens are injected into ovariectomized dogs. They regard it as a concomitant of endometrial growth in this species.

The metestrous bleeding in the cow is also a process of diapedesis with some denudation of the endometrial epithelium, particularly in the inter-caruncular areas (Weber *et al.* (1948)). The writers are not aware of any work, either in this species or in the guinea pig in which the time relations are much the same, that would give a clue to the cause of metestrous bleeding. The following experiments were devised to throw some light upon this problem in the cow.

Experimental

Altogether, 26 cows were used in the work. One had calved once and the rest were nulliparous. They were housed in a clean, warm barn and had access to a small pasture during the summer months. In winter they were turned out into an exercise lot for two hours each day.

The effect of ovariectomy. Fifteen cows were ovariectomized at various times after an observed heat. One, operated on during heat, bled 34 hours afterwards but the amount of blood was small. Another, operated on the day after heat, also bled 48 hours afterwards. The rest, two operated on during the third day after heat and the remainder at intervals spreading to 43 days, did not bleed. Apparently, at the time of heat, the impulse that culminates in bleeding has already been released. The ovary is the organ from which the impulse comes since spontaneous bleeding has not been observed in any of the ovariectomized cows, except for those described above.

The effect of acetylcholine and prostigmine. Since it has been shown in non-pregnant women that the injection of prostigmine methyl sulphate causes the onset of menstruation (Soskin *et al.* (1940)), this substance was injected into normal cows to test its effect. The theory behind the action is that it inhibits choline esterase, thus increasing the amount of acetylcholine, which causes uterine hyperemia. In order to reinforce the action of prostigmine, acetylcholine was also injected in some cases. One normal cow was injected with prostigmine methyl sulphate subcutaneously at eight, 13 and 19 days after heat, using 2,

4 and 3 cc., respectively, of a 1:2000 solution. Another was given 12 cc. by slow intravenous drip for six hours at 11 days postestrum, and a third, 16 cc. by the same method for eight hours at 20 days postestrum. None of these cows bled as a result of the injections. Two other cows that had been ovariectomized were brought into heat with stilbestrol and then were given 1 cc. of prostigmine methyl sulphate in one case, and 2 cc. in the other, together with .05, .1 and .15 g. of acetylcholine. No bleeding occurred in these cows nor in two similar ones treated with 3 and 6 cc. of prostigmine methyl sulphate alone.

The effect of diethylstilbestrol. In a first experiment four ovariectomized cows were given various doses of stilbestrol daily in quantities sufficient to bring them into heat. One of these cows bled. Upon repeating the injections she did not bleed.

Since microscopic bleeding is frequently observed in the normal cow it was considered that this might be taking place in the treated cows, so a new trial was made in which physiological doses (.255 mg. daily) of stilbestrol were injected into 15 ovariectomized cows until they came into heat. The injections were then stopped. During the injections and for a minimum of 11 days after, vaginal smears were taken, stained and examined for the presence of erythrocytes. The vaginal contents were also tested for blood using the benzidine method. Results from the smears and the benzidene test coincided in every case. In only one case was macroscopic bleeding observed and this was in the cow that had reacted similarly in the earlier experiment. Ten responded with microscopic bleeding, five did not. Five responded before or during heat and then gave smears without erythrocytes for a variable period. Responses after heat occurred in nine cows and the interval from heat varied from two to 14 days. There was a tendency for most positive responses to occur at one day before to one day after induced heat, and at nine and ten days after it. Two cows returned to estrus on the tenth and thirteenth days after the injections ceased. Since diethylstilbestrol is absorbed from subcutaneous injections rather slowly, as was shown by the renewed heats, the second wave of responses at nine to ten days cannot be regarded as a withdrawal effect.

Since microscopic bleeding was found in the injected cows with physiological doses it was decided next to try the effect of massive doses of stilbestrol in the hope that overt bleeding would be induced in consequence. One ovariectomized cow was given a single subcutaneous injection of 50 mg. She came in heat but did not bleed overtly though erythrocytes were present the day following the injection and were absent until the ninth and tenth days thereafter. Four other ovari-

ectomized cows were given subcutaneous doses ranging from .1 to 819 mg. in increasing daily injections over an 18 day period. No overt bleeding accompanied or followed these injections.

Five ovariectomized cows were treated with stilbestrol in 1.0–2.0 mg. doses daily for 118 days to study the effect of prolonged injections. None bled overtly.

The effect of other estrogens. Five ovariectomized cows were injected with .166 mg. of estradiol benzoate daily for 27 days. All came in heat and tended to remain in heat for several days. No macroscopic bleeding was seen in any of these cows.

Dienestrol was next tried, both subcutaneously and intravenously. This substance appeared to offer certain advantages since it is water soluble and is said to be absorbed and eliminated more rapidly than is stilbestrol. Doses ranged from .25 to 7.5 mg. Two ovariectomized cows were injected but neither showed either heat or overt bleeding. They did, however, show some estrogenic effects, such as increased flow of mucus and hyperemia of the vaginal mucosa. One ovariectomized cow was implanted subcutaneously with a pellet containing 460 mg. of dienestrol and the same amount of beta lactose. She neither came in heat nor bled overtly.

The effect of progesterone. Although the time relations of overt bleeding in the normal cow do not suggest that progesterone is involved it was decided to try the effect of this hormone. Seven ovariectomized cows were given 20 mg. of progesterone daily for six days, then six of them received 32 mg. daily for four more days and the seventh was continued at the 20 mg. level for 14 more days. None showed macroscopic bleeding either during or following the treatment.

Two ovariectomized cows were brought into heat with stilbestrol and were then given 35 mg. of progesterone daily for five days. One bled. This was the cow that had responded previously in the stilbestrol injections. A repetition with two other ovariectomized cows gave no response. Another trial was made in which 15 ovariectomized cows were given increasing doses of stilbestrol until they came in heat. During the six day period following estrus they were given increasing doses of progesterone, beginning with 15 mg. and rising to 90 mg. None bled overtly during or after the experiment. All showed microscopic bleeding at some time or other during or following the progesterone injections, but the distribution of the positive smears did not suggest that either injection or withdrawal was a favoring procedure. The progesterone injections did, however, increase the frequency of microscopic bleeding

when the results were compared with those obtained with stilbestrol alone.

It is possible that the hormonic conditions at the time of normal bleeding are such that estrogens are increasing and progesterone is decreasing, due to the growth of a follicle and the decline of a corpus luteum. To test the effects of such a combination progesterone was injected into 15 ovariectomized cows for 11 days at a decreasing rate from 90 to 15 mg. daily. On the third day stilbestrol injections were begun and continued until the cows came in heat on the seventeenth and eighteenth days. The dosage was increased from .025 to .5 mg. of stilbestrol during this time. No macroscopic bleeding occurred either during or after the treatment.

Because relatively large amounts of stilbestrol were required to overcome the estrus-inhibiting property of progesterone this aspect was explored further. Five ovariectomized cows were injected daily with 35 mg. of progesterone for 16 days. During the first 11, .5 mg. of stilbestrol were injected daily into each. The dose was then increased gradually until 2.4 mg. were given on the 17th day. No injections were made between the 18th and 28th days. On the 29th day a single injection of 1.0 mg. of stilbestrol was given. Only one cow had come in heat by the 17th day despite the relatively large amount of stilbestrol injected. Immediately after the injections on the 28th day, four of the five cows came in heat. Macroscopic bleeding did not occur during or following these injections.

In order to test the effects of these hormones given alternately in periods of short duration 15 ovariectomized cows were each given a single injection of .5 mg. of stilbestrol. All came in heat on the second day. On the third and fourth days each was injected with 35 mg. of progesterone, and on the sixth day with .255 mg. of stilbestrol. One cow bled overtly two days after she had come in heat to the second injection of stilbestrol.

Since it has been found by Engle *et al.* (1935) that small doses of progesterone given for five days during a course of estrogen injections will start bleeding in castrate monkeys the effect of this treatment in ovariectomized cows was tested. Five that were receiving 2.0 mg. of stilbestrol daily were given 32 mg. of progesterone daily for four days. Bleeding did not occur during or following this treatment.

Since plastic corrosion preparations of the uterine arterial system (Hansel and Asdell (1951)) have shown that castration atrophy is rapid in its onset, a few experiments were performed immediately after ovariectomy instead of waiting the usual five or six weeks before

treatment. Three heifers were ovariectomized and treatment was begun after two days. The first was given progesterone for three days, then estradiol, both alone and in combination with progesterone and with withdrawal periods. She did not bleed at any time. The second was treated similarly but she showed no overt bleeding until the 36th day when it occurred after fairly large doses of estradiol (1.6 mg.) during and following the injection of which 8 mg. of progesterone were given daily. The third also bled on the 48th day after similar treatment, but only to a very slight extent. The interval after ovariectomy in both these cases seems too long to enable us to draw the conclusion that a shorter lapse of time has affected the results.

Other experiments. An attempt was made to determine whether the blood of the normal cow contains a substance that causes bleeding. On the first and second days postestrum approximately 250 cc. of blood was drawn from a normal cow, defibrinated and injected into the jugular vein of an ovariectomized cow that had been brought into heat with estradiol following estradiol and progesterone treatment. Neither the normal nor the treated cow bled overtly. In a second pair the normal cow bled as usual and the treated one gave an unusual straw colored discharge from the fourth to the eighth day after the transfer. This vaginal discharge contained a few erythrocytes.

Injection of pituitrin into three ovariectomized cows was not followed by bleeding. Histamine, likewise, was without effect both in ovariectomized and in normal cows.

Another possible approach to the problem is to determine whether hormone injections will suppress an expected normal bleeding period. For this purpose six heifers with regular bleeding periods were chosen. The heat before treatment had been followed by bleeding in each case. Three were given 2 mg. of stilbestrol on the day of heat and on the two following days. Bleeding did not appear in two of them. One was given 1.0 mg. on each of these days. She did not bleed. One, given 1.6 mg. of estradiol on the day of heat and on the day following, also did not bleed. The last heifer received the same dose of estradiol on the day of heat and half this amount on the next day. She bled as usual. All these heifers bled normally after the next heat period. These results show that injected estrogen prevents the onset of bleeding in the normal cow.

In order to determine whether progesterone has the same effect, 32 mg. of this hormone were given to one cow on the day of heat and on the day after it during each of two cycles. She bled as usual each time. Two others were treated in like manner during one cycle and they, also,

bled as usual. Progesterone does not prevent the bleeding as does estrogen.

Discussion

Macroscopic bleeding two days after heat is not a constant phenomenon in the cow, though it does occur in the majority of heifers and in about half the cows. Complete success in reproducing it in treated ovariectomized cows cannot, then, be expected; there is an individuality component or the balance of hormones is very delicately poised. Even allowing for these possibilities our work has not given a completely satisfactory answer to the problem of its cause. The injections into normal cows show fairly clearly that estrogens, but not progesterone, suppress bleeding, presumably by maintaining the level of estrogens in the blood stream. It may, then, be regarded as an estrogen withdrawal phenomenon, though more work is needed before this view can be finally accepted.

The work with ovariectomized heifers and cows has given very limited positive evidence. The low level of estrogens in the cow, together with the slow rate of absorption of these hormones after subcutaneous injection in oil, may have obscured the issue. One cow has bled three times, once during progesterone injections and twice during stilbestrol injections. One responded to a sequence of stilbestrol, then progesterone followed by stilbestrol; two to stilbestrol and then progesterone in small amounts. In these it followed the cessation of stilbestrol injections and its further inhibition by progesterone. These limited successes cannot be interpreted one way or the other in view of the large number of failures, but they are not inconsistent with the estrogen withdrawal theory.

One link in the chain definitely lies in the ovary since bleeding ceases after ovariectomy. It is also evident, since bleeding follows if the operation is performed on the day of heat or the day after, that the ovary provides the stimulus and that removal of these organs at this time does not interfere with it. This evidence, too, is consistent with the estrogen withdrawal theory. Lack of success with injection technics may, perhaps, be linked with the fact that rapid regression of the uterine arterial system follows ovariectomy, though Hansel and Asdell (1951) have shown that administration of ovarian hormones prevents the usual depletion of endometrial arterioles that follows this operation. Another possibility is that the phenomenon is not closely linked with any of the known ovarian hormones, though the evidence does not support this idea.

We believe, in view of the evidence now available, and particularly from the fact that bleeding is much more frequent in heifers than in cows, that it is due to an estrogen withdrawal. The endocrine control in heifers may not be so well coordinated as it is in older cows. There may be a gap between the growth caused by the follicle and that caused by the developing corpus luteum. It is well known that the level of ovarian activity in young animals is not so great as it is in older ones, as witness the smaller number of follicles that ripen and rupture in young polytokous females. This suggests that in the growing animal the F. S. H. activity of the anterior pituitary is not so great as it is later in life. This may influence the level and continuity of action of estrogens. This view would link the bleeding with inter-menstrual hemorrhage in primates and particularly with the menstruation without ovulation that has been observed in monkeys and man. This is known to occur without the intervention of the corpus luteum and is taken as an indication of lesser F. S. H. activity, especially as it is found more frequently in young monkeys, and in summer when reproduction in this species is at a low ebb.

In contrast to macroscopic bleeding the extravasation of a few erythrocytes, enough to give the benzidine test, is easily produced experimentally. It has been encountered when either estrogens or progesterone are injected into the ovariectomized cow. When estrogens were injected this bleeding tended to occur at about the time of heat or around 10 days later. When progesterone was given all the treated cows responded with microscopic bleeding and they usually showed it for a much longer time than did the estrogen treated cows. These results suggest that this type of bleeding is the delayed result of progesterone treatment. The ten day interval between heat and microscopic bleeding, in normal and treated cows alike, is possibly a coincidence though this seems doubtful since the time relation holds approximately regardless of the dose given to treated cows. It may represent a reactive point as the level rises and then falls but this is doubtful for the same reason. It seems more likely that the interval represents a refractory period in the endometrium. It is therefore probable that the delay of 10 days in the normal cow before microscopic bleeding occurs is due to the intervention of a latent period.

Summary

1. Overt uterine bleeding follows if ovariectomy is performed during or one day after heat; not if the operation is performed at any other stage in the cycle.

2. Injections into ovariectomized cows of a variety of hormones, given in various combinations and dosages rarely cause overt bleeding.

3. The evidence favors the view that normal metestrous bleeding in the cow is due to estrogen withdrawal, although the cessation of estrogen injections does not cause overt bleeding in ovariectomized cows.

4. Microscopic bleeding in the ovariectomized cow occurs sometimes during estrogen injections. Then there is a latent period of about ten days after which they may bleed again.

5. With progesterone injections all cows showed microscopic bleeding.

6. Microscopic bleeding in the normal cow is probably due to progesterone and its occurrence at about ten days postestrum is probably due to the action of the latent period.

7. Prostigmine injections into normal cows are not followed by bleeding.

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THE FERTILITY OF COWS INSEMINATED AT VARIOUS INTERVALS AFTER CALVING[1]

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LITTLE evidence is available on the proper length of time after calving at which a cow should be rebred for optimum economy of the dairy cattle management operation. VanDemark and Salisbury (1950) have recently presented calving data from one herd mated naturally and have reviewed the published data on this question. Decision as to the proper time interval must be based upon the possible consequences of mating or insemination at the various intervals on such factors as the relative ease of conception, freedom from uterine infections and non-specific abortion, normal parturition in the subsequent calving, health and well-being of the calf produced, the effect of gestation on yield in the current and following lactation, total reproductive life of the cows involved, etc. All the evidence to date consists of survey data relating the length of the interval from calving to first service for the next pregnancy to one or another of the series of consequences. No controlled experiment seeking to answer the question has been conducted, nor have published surveys dealt with evidence from artificial insemination.

This paper presents data on the effect of the post-partum interval to first insemination on conception rate from that first service and from subsequent service in a cooperative artificial breeding organization.

Experimental

For the period from February 1, 1948, through January 31, 1949, the inseminator-technicians of the Southern Illinois Breeding Association recorded the dates of the previous calving for the cows they inseminated which had calved earlier. These records were made available to us for study. Only complete records showing calving dates, insemination dates, name and/or number of the individual cows involv-

[1] This paper is a part of a thesis to be submitted by the senior author to the Graduate College, University of Illinois, in partial fulfillment of the requirements for the degree of Master of Science in Dairy Science.

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ing no abnormal gestation periods were used. Data totaling 7,071 cows and 13,782 inseminations met these specifications. They were compiled by the calendar month in which the first insemination took place into 5-day time-interval classes from calving to first insemination. These data were combined into 20-day post-partum to first insemination time-interval classes to simplify the statistical analyses. The number of the bull used and the interval from first service to subsequent service, if any, were recorded. The basic data were non-return to service estimates of conception rate during a period of at least 180 days after insemination. These were tabulated for 12 calendar months by 11 time-interval classes, each representing a 20-day interval. The semen used during

TABLE 1. A COMPARISON OF THE PERCENT OF COWS CONCEIVING ON THE FIRST AND EACH SUBSEQUENT SERVICE WHEN MEASURED BY CALVES BORN AND BY NON-RETURN TO SERVICE IN NOT LESS THAN 180 DAYS AFTER INSEMINATION

	Service sequence								
	1	2	3	4	5	6	7	8	9+
Calves born natural mating[1]	55.8	47.6	47.9	42.6	41.4	42.6	38.5	37.5	100
Non-returns to service, artificial insemination	50.9	47.7	56.2	50.9	61.0	63.8	61.8	76.2	84.0

[1] Data from VanDemark and Salisbury (1950).

the period covered by these records was diluted with a 2.9 percent citrate-yolk diluter containing neither antibiotics nor chemotherapeutic agents. All bulls used in the stud at the time were represented in the basic data and careful studies indicated that the effect of bulls was distributed randomly throughout the post-partum to first insemination time-interval classes. The chi-square test and the analysis of covariance as described by Snedecor (1946) were used to determine the statistical significance of the differences shown in the data.

Results

The over-all 180-day non-return rate is shown in table 1. The data are shown as the percentage of inseminations resulting in non-return to service during at least 180 days for cows bred for the first time since calving, for the second time, etc., up to the ninth insemination, and are compared with data on calving rate from natural service. It can be seen that the non-return evidence in this study lacks validity after

more than three inseminations. It appears that cows failing to conceive on third insemination are not routinely returned for further inseminations, even though repeat services involved no additional charge. Therefore, only the evidence on first and second inseminations are reported; third services were used in this case only to determine the success of second inseminations.

Table 2 presents the evidence on the conception rate of the cows

TABLE 2. THE EFFECT OF THE LENGTH OF THE POST-PARTUM TO FIRST INSEMINATION INTERVAL ON THE CONCEPTION RATE OF 7,071 COWS

Post-partum interval to first insemination	No. obs.	Percent of total	Non-returns to first insemination	No. obs.	Non-returns to second insemination	Non-returns to maximum of two inseminations
		%	%		%	%
1– 20	24	0.3	20.8	19	57.9	66.6
21– 40	438	6.2	37.7	273	42.1	64.0
(41– 50	678	9.6	48.7	348	42.8	70.6)
(51– 60	1062	15.0	52.5	504	49.4	76.0)
41– 60	1740	24.6	51.0	852	46.7	73.9
61– 80	2182	30.9	51.2	1065	46.7	74.0
81–100	1410	19.9	51.5	684	48.2	74.9
101–120	623	8.8	54.6	283	49.5	77.1
121–140	306	4.3	50.7	151	51.7	76.2
141+	348	4.9	57.8	147	59.9	83.0
Total	7071		50.9	3474	47.7	74.3

inseminated for the first time during each of eight 20-day time intervals after parturition. The last four classes actually used in the statistical analyses were combined in the table. Also shown in parentheses in the table are the data for the two 10-day intervals, 41–50 and 51–60 days. The differences observed in conception rate on first service among the 20-day time intervals are highly significant statistically. The curve described is that of an asymptotic approach to an optimum. The practical limit of this approach was observed in the time interval of 51–60 days post-partum for first insemination, during which 52.5 percent of the 1,062 cows bred apparently conceived as measured by non-returns in not less than 180 days after first insemination. This level of conception for the 51–60 day interval was slightly higher than that for the next major class shown in table 2, 61–80 days, in which a 51.2 percent

conception resulted, but the difference was not statistically significant.

Also shown in table 2 are the number and conception rate of cows mated a second time after they had failed to conceive on first insemination. Of those cows which were first inseminated within 50 days after calving, but which did not conceive and were, therefore, reinseminated later, 43 percent conceived on second service. For all those cows reinseminated after a first insemination more than 50 days following calving, 48.8 percent conceived as indicated by non-return to service. This difference was not significant, the odds being $<9:1$ but $>19:1$ that the difference was not due to chance alone.

If a trend for fertility on second insemination to increase with the length of the interval from calving to first insemination does exist, it is

TABLE 3. THE AVERAGE INTERVALS IN DAYS BETWEEN CALVING AND FIRST INSEMINATION FOR 7,071 COWS AND BETWEEN FIRST INSEMINATION AND SECOND INSEMINATION AND BETWEEN ALL OTHER INSEMINATIONS FOR THOSE NOT CONCEIVING ON FIRST SERVICE

Post-partum interval to first insemination	No. cows	Mean days from calving to first insemination ± S.D.	No. cows	Mean days first to second insemination ± S.D.	No. intervals	Mean days between all other inseminations ± S.D.
days						
1–20	24	16.1± 3.7	19	34.6±19.8	8	24.8+14.6
21–40	438	33.0± 5.3	273	36.3±25.5	158	31.4±16.5
All	7071	78.4±35.0	3474	37.4±27.9	1828	34.6±22.1

not reflected in a change in the interval between first and second inseminations. As shown in table 3, there was no real difference in the intervals from the first to the second insemination for the two shorter post-partum to first insemination classes as compared with that for the total. Thus, it would appear that any carry-over effect from too early insemination after calving does not materially affect cycle length, but could possibly be due to infections or other disturbances resulting from insemination into uteri, the mucosa of which had not returned to normal following calving.

The summated results of the effect of the post-partum interval to first insemination on both the results of first and of second inseminations, regardless of the time between first and second inseminations are shown in the right-hand column of table 2. The trend in total conceptions from a maximum of two inseminations presented in the table is described by a linear regression on time. However, in the interval of 41–50 days

after calving, of 678 cows inseminated 70.6 percent conceived on a maximum of two services, whereas in the 51–60 day time-interval after calving, of 1,062 cows inseminated 76.0 percent conceived in a maximum of two inseminations. Thus, evidence was obtained to show that this sample of cows required a minimum of 50 days from calving to first insemination before relatively satisfactory conception results were achieved.

The percentage of cows falling in the various time-interval groups from parturition to first insemination did not follow any specific pattern and did not vary significantly from month to month. On the other hand, there were statistically significant differences among months in conception rate to first service. However, these differences reflect known changes in semen handling and insemination techniques not associated with seasonal changes. Thus, the true significance of the monthly differences in fertility level was obscured.

Discussion

It should be borne in mind that the data forming the basis of this study were obtained during a period in which the breeding association was rapidly expanding its operations. No antibiotics or chemotherapeutic agents were used in the diluted semen. Thus, the fertility level reported is lower than the current level resulting from stabilization of semen handling and insemination techniques by the organization. Also, the non-return estimates are based on a period of at least a 180-day interval after insemination, which fact alone reduces the mean several percentage units below the level obtaining on the basis of the usual 60–90 day non-return reports.

Nevertheless, the authors believe that the data presented provide a valid comparative estimate of the effect of the time interval after calving to first insemination on conception rate. If conception rate were the only item to be considered in determining the date at which a cow should be rebred after calving, on the basis of these data a minimum interval of 50 days would be recommended for cows in artificial breeding programs. However, VanDemark and Salisbury (1950) have emphasized that other items must be considered in determining the optimum date for rebreeding cows after calving. Certainly in view of the lowered conception rate reported here for both first insemination and for cows inseminated the second time which were first bred within 50 days after calving, an interval of less than 50 days from calving to insemination is not to be recommended.

Summary

A study was made of the fertility level of 7,071 cows inseminated at various intervals after calving. The results indicated that a minimum interval from calving to first insemination of 50 days was required for satisfactory fertility.

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HERITABILITY OF GRADE AND TYPE IN RANGE BEEF CATTLE[1]

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GRADE is one of the economic characteristics of cattle which must be considered in most cattle breeding operations. While quality enthusiasts may have ascribed to grade or type certain virtues that are non-existent, the cattle industry likely will continue to have certain quality standards. The success of breeders in producing cattle with the desired quality is determined largely by the heritability of the traits involved.

The marked changes in size, form, and quality of cattle that have taken place since the time of Robert Bakewell indicate that these traits are heritable. The results from grading up unimproved stock and of various cross-breeding operations likewise suggest a fairly high heritability. Knapp and Nordskog (1946) reported a preliminary estimate of heritability of weaning grade of Hereford cattle to be 53 percent. This estimate was later lowered to 28 percent (Knapp and Clark, 1950). Tyler and Hyatt (1948) found the official type rating of Ayrshire cattle to be 28 percent heritable. It is the purpose of this paper to report the heritability estimates gained from a study of two range herds and to give a preliminary report on the effectiveness of selection for type in cattle.

Experimental Material and Results

Records From Aberdeen-Angus Cattle[4]

The weaning grades of 715 calves and their dams were obtained in a herd of Angus cattle run on a semidesert range in northern Mexico. The calves were by 15 sires. The dams were sired by 10 known bulls

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TABLE 1. WITHIN YEAR MEAN SQUARES AND MEAN COVARIANCE OF WEANING GRADE SCORES FROM ANGUS CALVES AND THEIR DAMS

Source of Variance	Degrees of Freedom	Total Score			Lowness Score			Thickness Score			Smoothness Score		
		Dams	Calves	Products	Dams	Calves	Products	Dams	Calves	Products	Dams	Calves	Products
Total	708	3.22	4.53	.81	.355	.323	.081	.158	.199	.011	.290	.247	.020
Sires[1]	32	3.09	10.44	1.50	.250	.500	.031	.219	.281	.000	.375	.438	—.031
Within sires	676	3.04	4.25	.78	.359	.315	.083	.155	.195	.012	.289	.240	.022

[1] k=17.9

and one group of cows whose sires were unknown were treated as an eleventh sire group. The cows were hand bred and both parents of all calves used for the study were known.

The grading, which was begun in 1943, was done by one man. Each calf was given a numerical over-all score ranging from 70 to 83. Lowness, thickness, and smoothness each were given letter scores of A, B, or C. When the data were summarized, those letter scores were assigned the numerical values of 110, 100, and 90.

The data were classified by year, sire of calf, and sex of calf, and covariance of dam and calf scores was determined. There was no apparent difference due to sex so the two groups were combined. The mean squares and products for the four scores are shown in table 1.

TABLE 2. HERITABILITY ESTIMATE FOR WEANING SCORES. DATA FROM RANGE ANGUS HERD

Method of Estimating Heritability	Total Score	Lowness Score	Thickness Score	Smoothness Score
Intra-sire regressions of offspring on dam (x 2)	50.4	46.1	15.2	15.4
Intra-class correlation of calves Half-sib correlations (x 4)	30.1	12.7	9.6	17.6

Heritability estimates of the scores were calculated from (1) the intra-sire regression of calf on dam and (2) from the intra-class correlation of calves by the same sire. These are shown in table 2.

The highest heritability estimate was found in the over-all score. This was likely because the classification of lowness, thickness, and smoothness into three categories only was not refined enough for satisfactory analysis. There were fourteen categories on the scale for over-all grade. The estimates for over-all grade were 30 and 50 percent and are intermediate to the preliminary and revised estimates of the Miles City group (Knapp and Nordskog, 1946; Knapp and Clark, 1950).

The lowness score was based on length of leg and was primarily one of skeletal proportions. The estimates for this score were 13 and 46 percent. Estimates based on regression of offspring on dam for these two scores were substantially higher than those derived from intra-class correlation of half sibs. The higher estimates appear to be more in keeping with the selection experiment reported later in this paper.

Thickness and smoothness were undoubtedly more affected by factors influencing condition of calf. As would be expected, heritability estimates of these scores were generally lower, ranging from 10 to 18 percent.

Data from Hereford Cattle

The records used in this part of the study were the weaning grades of 1,257 calves and their dams on the experimental range of the New Mexico Agricultural Experiment Station. The calves were born from 1938 through 1950 and their dams from 1935 through 1947.

A committee of three or more people working independently graded the calves at weaning time when the animals averaged 205 days of age. The grades assigned originally were nine letter grades ranging from a low of C minus to a high of A plus. The grades from the committee were averaged and the grade nearest the average was recorded. For analysis, the letter grades were assigned numerical scores ranging from 50 for C minus to 130 for A plus, with an interval of 10 between adjacent grades.

The records were first classified by year, sire of calf, and sire of dam, and the data analyzed by variance and covariance methods. For the immediate study the classification by sire of dam was abandoned and the data grouped by sires within years. A summary of 13 years' records is shown in table 3.

The average heritability estimates from half sib correlations and from intra-sire regression on dam score were 24 and 23 percent, respectively. These estimates are substantially lower than for the corresponding over-all score in the preceding study on Angus cattle, and slightly lower than the revised estimate by Knapp and Clark (1950) of 28 percent, which was obtained from Hereford cattle.

One of the interesting features of these data was the variation in estimates for different years. When forage was scant because of drought, regression of calf score on dam was low, and in one year it was negative. During years when forage was average or above, regression varied from .18 to .22. That these yearly variations were highly significant is shown in table 3 (Snedecor, 1943). Whether the association with feed conditions was coincidental is not known but it raises again the question of relative adaptability under different conditions.

The intra-class correlation of half sibs also varied markedly during different years but apparently for a different reason. Prior to 1946 bulls were mated to random groups of cows except that closely related matings

TABLE 3. COVARIANCE ANALYSIS OF WEANING GRADE SCORES OF CALVES (y) AND THEIR DAMS (x).
Data from New Mexico Experimental Range

Source	df	Mean Squares and Products			Reg. Coef.	Errors of Estimate	
		Sx^2	Sy^2	Sxy		df	M.S.
Total	1256	155.7	106.7	30.2	.1941	....	.....
Years	12	1683.4	1648.6[1]	1344.0	.7984	....	.....
Within years	1244	141.0	91.8	17.5	.1244	1243	89.8
Sires in years[2]	88	346.0	155.8[1]	59.5	.1720	88	146.5[1]
Within sire and year	1156	125.3	86.9	14.3	.1145	1155	85.4
Yearly intra-sire regressions	....	.....	.....	.....	.....	12	192.3[1]
Deviations from yearly intra-sire regressions	....	.....	.....	.....	.....	1142	84.3

[1] Probability less than one percent.
[2] k=12.4. Heritability=4 x half sib correlation=24.0 percent.

and matings of animals with similar color defects were not usually made. During this period half sib correlations yielded a mean heritability of 16 percent. Beginning in 1946 the herd was split into three groups and a type-size experiment was started. One group of heavy cows was thereafter mated to the largest bulls. Another group of cows was selected for compactness and was mated to compact bulls. The remaining intermediate cows were mated to intermediate bulls. Since body proportions entered into the grade score (compact animals grading higher) the procedure had, to some degree, the effect of mating like to like rather than mating randomly. The mean heritability after beginning this mating system was 40 percent, reaching a high of 63 percent in 1950.

These results point out the need for random mating if half sib correlations are to be reliable in estimating heritability. The correlations obtained before 1946 were likely lower than would be obtained with a random sample of bulls, since the bulls used were selected for the same characteristics and were related. Half sib correlations after 1946 were probably higher than normal because of selective mating. However, the change in half sib correlation brought about by mating systems is indicative of a fair degree of heritability.

The Effectiveness of Selection for Compactness

The crucial test of the heritability of a character is the response to selection. The type-size experiment described in the preceding section provided an opportunity for observing the differences in body proportions of calves resulting from parents of different types. Since compactness was a major factor in determining grade, a preliminary report of this selection experiment was included.

The original selection of animals of different types was made from a herd in which all the animals were of similar breeding. The cow herd was sired by a group of related bulls from a small purebred herd owned by the station. The cattle were probably more uniform in breeding and phenotype than average commercial herds. The bulls used on these cows were from the same purebred herd as the sires of the cows. The calves dropped by first calf heifers in 1950 were the only second generation calves born, the remainder being from cows in the original selection.

The calves from different groups of cows showed marked differences in general conformation. Calves from compact cows were decidedly more compact than those from the large cows, while those from the

Steers from large cows. Steers from compact cows.

Figure 1. Representative steers from parents for large size and from parents bred for compactness. Note the shorter legs and bodies and smaller size of the compact steers.

medium cows were intermediate. The average grades of calves from large, medium, and compact animals were 104.1, 106.3 and 109.2, respectively.

Figure 1 shows pictorially the differences in type of steers from the large and compact groups. The pictures are of steers which were placed on feed in November, 1950, at weaning time and fed until May, 1951. The steers were of the same average age and were photographed on the same day. The differences observed in the photographs are considered

to be representative of those generally observed between calves from the two groups. The results of this selection experiment to date leave little doubt as to the higher heritability of general body proportions and size.

Discussion

Two possible reasons for the heritability estimate of over-all grade being higher from the Angus herd than from the Hereford might be suggested. First, only one person graded the Angus cattle, while a committee graded the Herefords. The range in grades by one individual is greater than the range of a committee grade. Thus, a committee grade has the advantage of being less subject to large errors, but has the disadvantage of reducing variation in scores. Also, individual scores are more likely to reflect variation of a single trait than that of a committee. Secondly, the Angus herd may not have been as highly selected at the beginning of the study as the Hereford herd. If a character is heritable, selection for that character would reduce its heritability within a group of animals. For this reason heritability estimates derived within relatively small groups of uniform animals do not necessarily give an indication of the general heritability of the character in the population as a whole (Lush, 1948).

The results from the selection experiment in the station herd thus far suggest a higher heritability of grade than the estimates derived from weaning scores. Since selection was made on general type rather than grade, which, in addition, includes factors such as condition and smoothness, the results are not directly comparable.

The results of this study along with that of the Miles City group indicate that the heritability of grade and type of beef cattle is about 30 percent or greater and that considerable progress in improving the quality of beef cattle can be accomplished by selection.

Summary

Weaning scores from 715 Aberdeen-Angus calves and their dams yielded heritability estimates for scores as follows, based on within-year regression of offspring on dam and half sib correlations: over-all weaning score, 50 and 30 percent; lowness score, 46 and 13 percent; thickness score, 15 and 10 percent; and smoothness score, 15 and 18 percent.

Records from 1,257 range Hereford calves and their dams resulted in heritability estimates for grade of 24 and 23 percent, based on half

sib correlation and intra-sire regression of offspring on dam, respectively.

Preliminary results showed selection for compactness in Hereford cattle to be highly effective, indicating a relatively high heritability of body proportions.

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RELATIONSHIPS OF MEASUREMENTS OF LIVE ANIMALS TO WEIGHTS OF WHOLESALE CUTS OF BEEF[1]

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A PAUCITY of knowledge seems to exist concerning the use of bodily measurements for estimating and predicting the weights of various parts of beef cattle. Study of the relationships among weights and other measurements could be of practical importance in the evaluation and selection of animals. The establishment of the validity of certain teachings and opinions associated with judging practices might also result from such studies.

Measurements have been used in beef cattle for the estimation of bodily weights (Johnson, 1939), studies of changes during fattening periods (Hultz, 1927 and Lush, 1928), rate and efficiency of gain (Black *et al.*, 1938 and Kohli *et al.*, 1951), and carcass grade and dressing percent (Cook *et al.*, 1951). Various phases of record of performance testing in beef cattle, including measurements, have been reviewed by Mason (1951). Hetzer *et al.* (1950) studied the relationships of bodily measurements and the combined yield of five cuts of swine, but no reports were found concerning the relationships among measurements of live beef animals and the weights of wholesale cuts yielded by the animals measured. This present study was made to ascertain the relationships between measurements made on the live beef steers and the weights of major wholesale cuts.

Materials and Methods

Fifty beef-type steers purchased on the Baltimore market by a meat packing concern of that city were made available for this study. These animals were considered as "normal run" cattle that ranged in weight from 800 to 1,445 pounds and in grade from choice to medium, although most were choice and good.

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Linear measurements were taken by placing each animal in a 3 x 7 foot holding pen that had a hard, level floor. Although confined, the animals stood in a normal position free from restraint. A steel tape and a large metal caliper both of which were graduated in feet and hundredths of a foot were used for all measurements.

Measurements were based on the points of cutting the carcass as well as common reference points used in visual appraisal. The following were secured by stretching a steel tape from point to point over the skin (hair) surface of the animal (the tape passed straight across concave portions of the body): (a) Withers (highest point on a medial plane over the spinous processes of the second and third thoracic vertebrae or highest point on the mid-line in the region of the suprascapular cartilages) to the middle of the knee. (b) Withers to the extreme lower edge of the fore flank. (c) Withers to a point between the dorsal processes of the fifth and sixth thoracic vertebrae (5/6 rib). (d) Medial dorsal plane distance from 5/6 rib to a point between the dorsal processes of the 12th and 13th thoracic vertebrae (12/13 rib). (e) Length from 12/13 rib to a medial point midway between the anterior skin surfaces of the hock bones (tuber coxae). (f) Distance 12/13 rib to mid-rump, mid-rump being the point over the spinous process of the first coccygeal vertebra or one-half the distance from the hook to pin (posterior skin surface over the tuber ischii). (g) Pin to mid-rump. (h) Hook to mid-rump was secured by subtracting the length of the 12/13 rib to hook from that of the 12/13 rib to mid-rump. (i) Mid-rump to point of hook (tuber calcis). (j) Mid-rump to lower edge of the rear flank. (k) Circumference of heart girth taken just behind the point of the elbow (olecranon). (l) Circumference of paunch taken at widest point of the paunch. (m) Circumference of the rear flank taken just anterior to the stifle (patella). (n) Depth of twist from lower aspect of the anus to a point where the hind legs became separate entities—a ruler was held horizontally at the latter point so the distance measured was essentially perpendicular from the anus.

Projected distances were secured by using a metal caliper graduated to one-hundredth of a foot. (o) Depth of chest from withers to floor of chest just behind the elbow. (p) Width of shoulders at widest point. (q) Width of crops at approximately the eighth rib and 4 inches below the level of the back. (r) Width of loin midway between the 13th rib and hook. (s) Width of hooks from lateral aspect of one hook to the corresponding point of the other. (t) Width of thighs at the widest point of the lateral sides. (u) Width of pins measured from the center of the

posterior surface of one pin bone to the same point on the other. (v) Horizontal distance from stifle to posterior surface of hind leg (stifle to "bulge"). (w) Depth of rib taken from the level of the back to a point 9 inches down as measured on the surface of the body. (x) Depth of loin from the level of the back to the lower edge of the loin as determined by palpation.

Live weights were secured by using a livestock scale graduated in 5-pound units. Slaughter grade was estimated on the basis of the standards in the U.S.D.A. Bulletin 1360 (Burk and Whalin, 1942). For purposes of calculation, the grades were classified into three subgrades (e.g. choice plus, choice, and choice minus) and were given numbers where choice-plus was scored as nine, choice as eight, and so forth down to medium-minus as one. Only one measurement was taken for any distance, weight or grade. Replicated measurements may have added accuracy and insured reliability of the measures, but facilities did not allow the taking of duplicate or triplicate measurements. The animals were slaughtered soon after measuring and weighing.

The weights of the wholesale cuts represented the combined weights of each cut taken from each side of the carcass. All cuts were weighed to the nearest quarter pound. Weight of the trimmed loin was secured by adding the weights of the short loin and sirloin butt. The weight of the rough loin was secured by adding the weights of the trimmed loin and the loin trim (flank, kidney knob and hanging tenderloin). Brisket and arm chuck were added to give the weights of the cross cut. Carcasses were cut according to the procedures given by the U. S. Office of Price Stabilization (Anonymous, 1951).

Analysis of the data was made according to Snedecor (1948) and Peters and Van Voorhis (1940). Modifications in the calculation of multiple correlation coefficients by use of the Doolittle Method were suggested by Dr. T. G. Andrews (personal communication). Zero order correlations were carried to ten decimal places for calculating the multiple correlations in order to get maximum accuracy in the first one to three decimal places (Hotelling, 1943). The multiple correlations will be reported to three decimal places and should be accurate within the limits of the accuracy of the original measurements.

Results

Means, standard deviations, and coefficients of variation for the measurements and weights are given in table 1. In general, the smaller coefficients of variation are associated with the measurements of cir-

TABLE 1. MEANS AND STANDARD DEVIATIONS OF MEASUREMENTS TAKEN ON LIVE STEERS AND WHOLESALE CUTS OF THEIR CARCASSES

Measurement or Cut	Mean	Standard Deviation	Coefficient of Variation	Units of Measurement
			(percent)	
Live weight	1118.80	158.18	14.1	pounds
Slaughter grade	5.82	1.30	22.4	[1]
Withers to fore flank	2.32	0.15	6.6	feet
Withers to knee	3.45	0.18	5.3	feet
Withers to 5/6 rib	0.51	0.11	22.1	feet
5/6 rib to 12/13 rib	0.91	0.15	16.1	feet
12/13 rib to hook	1.13	0.12	10.8	feet
12/13 rib to mid rump	1.96	0.13	6.5	feet
Hooks to mid rump	0.83	0.11	13.1	feet
Pin to mid rump	0.85	0.09	10.5	feet
Mid rump to point of hock	3.30	0.15	4.6	feet
Mid rump to rear flank	2.49	0.13	5.2	feet
Circumference of heart girth	6.33	0.33	5.1	feet
Circumference of paunch	7.06	0.32	4.5	feet
Circumference of rear flank	6.17	0.33	5.3	feet
Depth of chest	2.46	0.15	6.1	feet
Width of shoulders	1.60	0.13	8.1	feet
Width of crops	1.02	0.10	9.5	feet
Width of loin	1.22	0.11	9.2	feet
Width of hooks	1.60	0.11	6.8	feet
Width of thighs	1.65	0.12	7.2	feet
Width of pins	0.43	0.08	18.5	feet
Depth of twist	1.25	0.13	10.2	feet
Stifle to bulge	0.94	0.09	9.9	feet
Depth of rib	0.71	0.10	13.9	feet
Depth of loin	0.44	0.07	15.5	feet
Weight of rib	53.07	8.87	16.7	pounds
Weight of cross cut	223.51	33.42	15.0	pounds
Weight of short loin	49.35	8.49	17.2	pounds
Weight of sirloin butt	55.71	8.11	14.6	pounds
Weight of trimmed loin	105.10	16.15	15.4	pounds
Weight of rough loin	151.92	26.75	17.6	pounds
Weight of round	155.52	21.67	13.9	pounds

[1] Slaughter grade 5 is equivalent to "good" and 6 to "good plus" (old standard).

cumference and those involving the long bones. Length measurements as taken along the dorsal mid-line have larger coefficients. These may have been due to actual variations among the animals or to errors of measurement due to a lack of definiteness of point of reference even though those points are readily distinguishable. The average length of the animals from withers to pin bones was 4.21 feet with a standard deviation of 0.21 foot and a coefficient of variation of 5.1 percent which is

TABLE 2. SIMPLE CORRELATIONS BETWEEN THE WEIGHTS OF CUTS AND ASSOCIATED MEASUREMENTS AND BETWEEN OTHER MEASUREMENTS AS INDICATED*

		(1)	(2)	(3)	(4)	(5)	(7)	(8)	(10)	(12)	(13)	(14)
Live weight	(1)		.27	.05	.75	.53	.79	.38	.37	.75	.51	.78
Slaughter grade	(2)			−.13	.48	.26	.30	−.03	−.15	.22	.38	.43
12/13 rib to hooks	(3)				.13	−.11						
Width of loin	(4)					.38	.76	.13	.24	.59	.57	.77
Depth of loin	(5)						.41	.45	.40	.37	.42	.53
Wt. of short loin	(6)	.89	.03	.14	.72	.46						
Width of hooks	(7)							.26	.44	.74	.46	.77
Hooks to mid-rump	(8)											
Wt. of sirloin butt	(9)	.92	.03		.72	.46	.76	.31				
12/13 rib to mid-rump	(10)									.37	.19	.23
Wt. of trimmed loin	(11)	.93	.03		.73	.47	.76		.36			
Mid-rump to rear flank	(12)										.52	.79
Circumference of paunch	(13)											.63
Circumference of rear flank	(14)											
Wt. of rough loin	(15)	.92	.04		.70	.51	.75		.33	.70	.01	.79

		(1)	(2)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)
5/6 rib to 12/13 rib	(16)	.02	−.24	−.20	.02	.03						
Width of crops	(17)	.68	.37		−.13	.72						
Depth of rib	(18)	.09	.27			.02						
Weight of rib	(19)	.92	.03									
Withers to fore flank	(20)	.71	.40	.39				.63	.10	.74	.48	.49
Withers to knee	(21)	.76	.08	.45					−.02	.75	.37	.58
Withers to 5/6 rib	(22)	.12	.14	.22						.20	.37	.08
Circumference of heart girth	(23)	.93	.32	.73							.56	.75
Depth of chest	(24)	.55	.17	.24								.30
Width of shoulders	(25)	.76	.29	.78								
Weight of cross cut	(26)	.94	.03	.62			.68	.70	.11	.87	.53	.78

		(27)	(28)	(29)	(30)	(31)	(32)	(33)
Live weight	(1)	.53	.70	.78	.23	.61	.51	.91
Slaughter grade	(2)	.45	.17	.40	.15	.18	.03	.27
Width of hooks	(7)	.55	.57	.78	.00	.68	.45	.79
Mid-rump to rear flank	(12)	.48	.73	.71	.08	.63	.34	.76
Pins to mid-rump	(27)		.42	.52	.17	.31	.17	.47
Mid-rump to point of hock	(28)			.63	−.07	.67	.45	.72
Width of thighs	(29)				.02	.51	.41	.77
Width of pins	(30)					.06	.03	.14
Depth of twist	(31)						.38	.68
Stifle to bulge	(32)							.49
Wt. of round	(33)							—

* Correlations of 0.28 are significant at the 5 percent level ($P = <0.05$) and 0.36 at the 1 percent level ($P = <0.01$).

TABLE 3. STANDARD PARTIAL REGRESSION COEFFICIENTS (BETAS) AND MULTIPLE CORRELATION COEFFICIENTS (R) FOR VARIOUS COMBINATIONS OF MEASUREMENTS PERTAINING TO THE CROSS CUT (NO. 26)

	Length, Withers to			Width of		Depth of Chest (24)	Circum. Heart Girth (23)	Live Weight (1)	Slaughter Grade (2)	R**	R^2
	foreflank (20)[1]	knee (21)	5/6 rib (22)	shoulders (25)	crops (17)						
(a)	0.201	−0.160	0.020	0.278	−0.033	0.032	−0.022	0.823	−0.338	0.993	0.986
(b)		−0.117		0.203			0.068	0.892	−0.290	0.983	0.967
(c)		−0.013		0.162			−0.098	0.919		0.947	0.897
(d)		−0.007		0.334			0.719		−0.303	0.932	0.869
(e)				0.298			0.643			0.888	0.788

[1] Number in ( ) indicates number in table 2.
** All correlations statistically highly significant (P=<0.01).

TABLE 4. STANDARD PARTIAL REGRESSION COEFFICIENTS (BETAS) AND MULTIPLE CORRELATION COEFFICIENTS (R) FOR VARIOUS COMBINATIONS OF MEASUREMENTS PERTAINING TO THE RIB (NO. 19)

	Depth of Rib (18)[1]	Length 5/6–12/13 Rib (16)	Live Weight (1)	Slaughter Grade (2)	Width of Crops (17)	R**	R^2
(a)	0.076	0.001	0.785	—0.325	0.315	0.968	0.937
(b)		0.001	0.808	—0.298	0.280	0.966	0.932
(c)			0.808	—0.298	0.279	0.966	0.932
(d)			0.794		0.180	0.925	0.855
(e)				—0.274	0.817	0.760	0.578

[1] Number in ( ) indicates number in table 2.
** All correlations statistically highly significant (P=<0.01).

similar in magnitude to the circumference and long bone measurements. All weights had about the same coefficient of variation. The slaughter grade numbered five was equivalent to a "good" grade and number six designated a "good plus".

Simple correlations between the weight of wholesale cuts (items 6, 9, 11, 15, 19, 26, and 33) and the measurements pertaining to each cut are shown in table 2 along with intercorrelations of all measurements involved for any particular cut. Most of the correlations between weight of cut and live weight of the animals were about 0.9 while those involving slaughter grade were very low and seldom statistically significant.

In general, the depth and width measurements were usually more highly correlated with the weight of the cut than were length measurements as taken along the dorsal mid-line.

TABLE 5. STANDARD PARTIAL REGRESSION COEFFICIENTS (BETAS) AND MULTIPLE CORRELATION COEFFICIENTS (R) FOR VARIOUS COMBINATIONS OF MEASUREMENTS PERTAINING TO THE TRIMMED LOIN (NO. 11)

	Width of Loin (4)[1]	Width of Hooks (7)	Depth of Loin (5)	Live Weight (1)	Slaughter Grade (2)	Length 12/13 rib to mid-rump (10)	R**	R^2
(a)	0.267	0.082	0.059	0.767	—0.360	—0.104	0.973	0.948
(b)	0.064	0.035	—0.040	0.864		0.023	0.930	0.865
(c)	0.355	0.412	0.155			0.030	0.810	0.656
(d)		0.182	0.043	0.882	—0.290	—0.108	0.963	0.926
(e)		0.066	—0.040	0.891		0.015	0.929	0.863
(f)		0.800	0.282		—0.303	—0.150	0.821	0.673
(g)		0.130	0.005	0.892	—0.251		0.959	0.919
(h)		0.072	—0.035	0.890			0.929	0.863
(i)		0.678	0.193				0.777	0.604

[1] Number in ( ) indicates number in table 2.
** All correlations statistically highly significant (P=<0.01).

TABLE 6. STANDARD PARTIAL REGRESSION COEFFICIENTS (BETAS) AND MULTIPLE CORRELATION COEFFICIENTS (R) FOR VARIOUS COMBINATIONS OF MEASUREMENTS PERTAINING TO THE SHORT LOIN (NO. 6)

	Length 12/13 rib to hook (3)[1]	Live Weight (1)	Slaughter Grade (2)	Width of Loin (4)	Depth of Loin (5)	R**	R^2
(a)	0.026	0.712	−0.322	0.316	0.043	0.933	0.871
(b)	0.087	0.810		0.098	−0.002	0.894	0.799
(c)	0.002		−0.429	0.827	0.255	0.835	0.698
(d)	0.083			0.620	0.231	0.750	0.563
(e)		0.809		0.116	−0.019	0.890	0.792
(f)				0.637	0.216	0.746	0.557

[1] Number in ( ) indicates number in table 2.
** All correlations statistically highly significant (P=<0.01).

Tables 3 through 8 show the standard partial regression coefficients and multiple correlation coefficients for various combinations of measurements associated with each cut. Use of all measurements indicates the greatest possible ability to estimate accurately the weight of each cut using the items given. Deletion of items (lines b, c, d, etc. in the tables) was done in order to ascertain more completely the actual effect of specific measurements as well as to find out the probable minimum number of items that could be used and still get reliable results. The measurements (and the associated partial regression coefficients) for the rough loin were length, mid rump to rear flank (−0.150), width of hooks (0.105), circumference of rear flank (0.402), live weight

TABLE 7. STANDARD PARTIAL REGRESSION COEFFICIENTS (BETAS) AND MULTIPLE CORRELATION COEFFICIENTS (R) FOR VARIOUS COMBINATIONS OF MEASUREMENTS PERTAINING TO THE SIRLOIN BUTT (NO. 9)

	Width of Loin (4)[1]	Width of Hooks (7)	Depth of Loin (5)	Live Weight (1)	Slaughter Grade (2)	Length hooks to mid-rump (8)	R**	R^2
(a)	0.239	0.049	0.022	0.791	−0.317	−0.039	0.962	0.925
(b)	0.563	0.364	0.173		−0.388	0.048	0.873	0.763
(c)	0.252	0.047	0.008	0.775	−0.316		0.961	0.924
(d)	0.556	0.375	0.194		−0.395		0.872	0.761
(e)	0.045	0.063	−0.048	0.862			0.923	0.851
(f)	0.333	0.443	0.149				0.802	0.643
(g)		0.135	0.026	0.908	−0.265	−0.092	0.955	0.911
(h)		0.739	0.212		−0.244	0.013	0.806	0.650
(i)		0.139	−0.009	0.883	−0.248		0.951	0.905
(j)		0.082	−0.049	0.881			0.922	0.851
(k)		0.140		0.878	−0.249		0.951	0.905
(l)		0.683	0.178				0.772	0.597

[1] Number in ( ) indicates number in table 2.
** All correlations statistically highly significant (P=<0.01).

TABLE 8. STANDARD PARTIAL REGRESSION COEFFICIENTS (BETAS) AND MULTIPILE CORRELATION COEFFICIENTS (R) FOR VARIOUS COMBINATIONS OF MEASUREMENTS PERTAINING TO THE ROUND (NO. 33)

	Length				Width						
	Mid-rump to point of hock (28)[1]	Pin to mid-rump (27)	Mid-rump to rear flank (12)	Stifle to bulge (32)	Hooks (7)	Thighs (29)	Depth of twist (31)	Live Weight (1)	Slaughter Grade (2)	R**	R^2
(a)		—0.058	0.088	0.014	0.071	0.075	0.148	0.662	0.013	0.933	0.871
(b)		—0.053	0.085	0.013	0.070	0.081	0.150	0.661		0.933	0.871
(c)		0.018	0.241	0.129	0.188	0.287	0.203		—0.006	0.874	0.764
(d)	0.058		0.058	0.012	0.075	0.063	0.136	0.632		0.933	0.870
(e)		0.016	0.242	0.130	0.188	0.285	0.203			0.874	0.763

[1] Number in ( ) indicates number in table 2.
** All correlations are statistically highly significant (P=<0.01).

(0.747), slaughter grade (—0.368), depth of loin (0.047), and length 12/13 rib to mid-rump (—0.103) ($R=0.975$, $R^2=0.950$). Various measurements were not studied by deletion in the case of the rough loin as the other three segments of the loin were of more direct and practical interest.

Discussion

The magnitude of the correlations secured in this study indicates that the actual weights of the parts of live beef-type animals from which wholesale carcass cuts are derived can be predicted with considerable accuracy by the use of bodily measurements, at least for the kind of cattle used in this study.

The greatest accuracy of prediction was found for the cross-cut wherein 98.6 percent of the total variations (R^2) in the weights of the cuts was accounted for by use of nine measurements. Lowest estimableness was in connection with the short loin and round where the degree of accuracy was 87.0 percent. The variation in the weights of the animals contributed greatly to the magnitude of the multiple correlation (R) and R^2 values as most of the weights of cuts were correlated in the neighborhood of 0.9 ($r^2=0.81$) with live weight. The significance of the use of measurements in addition to live weight may be measured by the increase in R^2 values over the r^2 for weight of cut and live weight (see table 2 for the latter correlations). This varies considerably from cut to cut, but in each instance one or more sets of measurements is better than live weight alone. Certain combinations of measurements in lieu of using live weight do about as good a job as live weight used alone—line d of table 3. In other cases, the addition of measurements to weight has little if any value: lines b, e, and h, table 5 (correlation of weight of trimmed loin and live weight was 0.927); lines b and e, table 6; and lines e and j, table 7.

For practical purposes, the sets of measurements in lines a and b of table 3; a, b, and c of table 4; a, d, and g of table 5; a of table 6; a, c, g, i, and k of table 7; and a and b of table 8 may be considered for use in predicting the weights of the cuts. The inclusion of live weight may be open to some questions as it is obvious that other things being equal, the more the animal weighs the more the parts should weigh. Another method of attacking this present problem would have been the use of animals all of the same weight. This was not possible under the conditions of this study and it is a situation that would seldom, if ever, be met under practical conditions. It is true that in many instances it

may be impossible to weigh animals on a scale; however, in some cases live weight may be estimated rather closely by an experienced person, or the weight can be estimated with reasonable accuracy by using bodily measurements. Many times inaccuracies in estimating live weight would be of less importance than the omission of live weight from the series of measurements used to predict the weight of the cuts. Live weight (and slaughter grade) has been omitted from the series of measurements for each cut to show the effect of such deletion for the information of any who may be of the opinion that live weight (or slaughter grade) should be excluded.

Removal of slaughter grade as a measure caused a significant lowering of the multiple correlation in all cuts but the round and, therefore, should be considered as an inclusion in the set of measurements. Length measurements as taken along the back of the animal seemed to contribute little to the accuracy of the prediction of the cut as deletion of these measurements caused no or little change in the R values for the rib, trimmed loin, short loin, or sirloin butt and contributed relatively little to the cross-cut or round when live weight was included as a measurement. Width and depth measurements seemed to be of more importance, in general, than length measurements taken along the back. Depth of rib was of negligible importance, however, in estimating the weight of the rib. That measurement was difficult to secure accurately, and under the conditions of this study may well be eliminated as a measure if live weight is used.

Some sets of measurements include items that may seem to be duplications in a certain sense such as the distance from the withers to the knee and fore flank, depth of chest and circumference of heart girth, and the width of the shoulders and crops in the case of the cross-cut or the width of the loin and hooks in the loin measurements. A number of these supposed duplications really measure different basic anatomical structures. A part of the size of the correlations between these possible duplicating structures as shown in table 2 is due to the correlations between these structures and the live weight. When the effects of the variations in live weight are eliminated from both variables by use of partial correlations, the size of the correlation from simple to partial is reduced from 0.63 to 0.20 for the lengths of withers to fore flank and withers to knee, 0.56 to 0.17 for depth of chest and circumference of heart girth, 0.78 to 0.56 for width of shoulders and crops, and 0.76 to 0.40 for width of loin and hooks. For most of the cuts where some apparent but perhaps not real duplication is indicated in the sets of

measurements, new calculations omitting all or most of these items are presented.

The effect of any one item in a series of measurements, which lead to a specific R and R^2, may be secured independently from the variation in the other items in the series by multiplying the beta value by the ratio of the standard deviation of the weight of the cut divided by the standard deviation of the item. This calculated number is, of course, the partial regression coefficient in terms of the actual units (feet or pounds) used to measure the animals. The magnitude of the beta does not indicate its actual importance as the ratios of the standard deviations might vary considerably.

For example, the first two betas of similar magnitude noted in table 3 are the ones for withers to 5/6 rib (0.02) and circumference of heart girth (—0.022). The standard deviations of the weight of the cross-cut and the two measurements are respectively 33.42, 0.1125, and 0.3251 (table 2) and the ratios are 297.07 and —102.8 which when multiplied by the respective betas equal 5.94 and —2.26. When these answers are divided by 0.01 they indicate that for each change in 0.01 foot in the length from withers to 5/6 rib, the weight of the cross-cut will change 0.0594 pound independent of changes in any of the other measurements used in line a of table 3, and that the change in weight of the cross-cut will be —0.0226 pound for each shift in 0.01 in the circumference of the heart girth independent of changes in any of the other indicated items on that line (a).

One of the objectives of this study was to see if it would be possible to establish the validity of the use of measurements for predicting the weights of bodily parts with the view in mind that more objective methods might be secured for the selection of breeding stock. The animals used in this study were steers within certain weight and slaughter grade limits established by the availability of animals. The correlations secured perhaps may be indicative of those that might be secured for animals of different sexes, ages, grades, and breeding. Before any definite recommendations can be made, it would be necessary to repeat this work on various groups of animals falling within specific ranges of sex, weight, slaughter grade, etc. It may be well true that different sets of equations may be necessary not only for different groups within the English breeds of beef animals as a whole (or even within breeds) but also for Brahman and Brahman-cross animals. The deduction of one formula for all types of cattle was not one of the objectives of this study and the results should not be so interpreted.

It is for this reason that regression equations have not been presented.

The correlations and other results secured from the steers used in this study present some interesting points for consideration from the standpoint of judging practices. A review of table 2 indicates that the rough loin and its various segments (trimmed loin, short loin, and sirloin butt) are correlated to essentially the same degree with various linear measurements (4, 5, and 7), live weight, and slaughter grade. Evaluation of one of these measurements in connection with one portion of the loin seems to suffice for the estimation of other portions. It is of further interest that the different parts of the loin represent different anatomical structures. Even under that situation, the correlations are, for practical purposes, interchangeable.

The beta values indicate the necessity for care in observation and exactness of emphasis of different dimensions of animals during judging. The amount of emphasis to be placed on any one dimension in estimating the weight of a cut can vary widely depending upon the number of different measurements being considered at any one time. As mentioned previously comparison of beta values on an absolute basis may lead to erroneous conclusions. If the beta values are properly weighted by the ratio of associated standard deviations, these comparisons of beta values may be correct and useful. The following example will illustrate: The ratio of the standard deviation of the weight of the cross-cut divided by the standard deviation of the circumference of the heart girth was previously shown to be —102.8. That figure is a constant for this particular set of data.

It is indicated from table 3 that when the combinations of measures shown in lines a, b, and c are used, little attention need be paid to variations in the circumference of the heart girth. These three beta values are all small and when multiplied by the ratio of the standard deviations, the answers are small. In contrast, the beta for circumference of heart girth shown on line e of table 2 is relatively large. This indicates that when only the two items of line e are considered, a great deal of emphasis should be placed on differences in the circumference of the heart girth.

Persons are apt to shift in the number of points at which they look during the judging of a group of animals or from one judging period to another. These shifts are not always accompanied by proper mental readjustments of proportionate values. This may lead to discrepancies which might be avoided if proper re-evaluations of the relative weighting

of any one point or dimension is made in reference to the number of points being considered at a given time.

Examples similar to the above could be cited for the other cuts. It is true that the kind and number of animals used in this study are somewhat limited, but the results do indicate some implications which are not always obvious to many who rely solely on visual methods for the evaluation of beef or other types of live stock. The methods presented as well as others that could be used indicate that further exploration of the basic factors involved in evaluating animals might be highly desirable.

Little difficulty was experienced in actually securing the measurements even though the animals were not halter-broken. Initial behavior of the steers indicated that they had been kept in dry-lot or on pasture prior to arrival at the stock yards and were not accustomed to handling. Within a few minutes after being placed in the holding pen, the steers stood quietly and in a "normal" position. The only measurements that were difficult to secure at times were those taken around the face (not reported here). In general, about 20 minutes were required per animal to place it in the holding pen, secure 36 linear measurements, estimate slaughter grade, secure live weight and paint brand for identification at slaughter time. The animals were not injured as a result of being measured. All carcasses were carefully checked for bruises which could have resulted from treatment in connection with this work but no such bruises were observed.

In view of the experience to date, it appears that measurements may be secured on most beef cattle of the English breeds except those that are extremely nervous. No attempts were made to measure Brahman or animals of part Brahman breeding.

Summary and Conclusions

Multiple correlations were used to study the predictability of the weights of wholesale cuts of carcasses by use of bodily measurements taken on 50 beef type steers prior to slaughter. The steers ranged in weight from 800 to 1,445 pounds (ave. 1118 pounds) and graded from choice to medium (mostly good and choice). Multiple correlation values secured for the various cuts were cross-cut, 0.99; rib, 0.97; rough loin, 0.98; trimmed loin, 0.97; short loin, 0.93; sirloin butt, 0.96; and round, 0.93. The size of the multiple correlations secured by various combinations of measurements for each cut was also studied. The

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practicability of securing measurements and the possible use of measurements as a possible objective method for assisting in the selection of breeding animals were discussed.

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EFFECT OF FATTENING UPON CERTAIN CHARACTERISTICS OF CONNECTIVE TISSUE IN AGED COWS[1]

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LIGHTFOOT and Coolidge (1949) showed with guinea pigs that there was a significant increase in the percent collagen with age. They also concluded that fasted animals at the growing age continued to synthesize collagen at the same rate as well fed animals. Spencer *et al.* (1937) found a rapid development of fibrous tissue in rabbits due to nutritional muscle dystrophy. Dystrophic rabbit muscle contained 2.0 to 2.5 times more collagen than the normal control rabbits. Ritchie (1922) in his work to determine the nitrogen distribution in beef flesh as affected by age and nutrition found that the total soluble nitrogen increased as the animals became fatter. The amino acid and extractive nitrogen content increased with the plane of nutrition; however this fraction again decreased with very fat animals due to the dilution with fat.

Since other investigators had found changes taking place in the connective tissue content of muscle due to variations in the nutritional level of small animals, it was felt that this type of study was pertinent to the meat animal field. This was largely based on the fact that tenderness in meat is primarily determined by the kind and amount of connective tissue. In addition tenderness is the most important quality factor contributing to the palatability of meat.

Procedure

This investigation was designed to study the effect of fattening on characteristics which may be associated with tenderness in meat. Two experiments were made, using in each, eight aged Holstein cows weighing approximately 1100 pounds and grading U. S. Cutter. In each experiment the cows were fed a ration made up of 10 parts oats, 8 parts corn, 2 parts linseed oil meal and .2 part salt in addition to corn silage and mixed hay. In the first experiment (summer 1949) the cattle consumed varying quantities of feed depending on their appetite, while in the

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second trial (summer 1950) the cow with the least appetite determined the amount fed in an attempt to make the rate of gain more nearly constant. The cattle in the first experiment were fed 67 days and the second group 74 days.

At the beginning of the feeding period muscle samples were taken for analysis by biopsy from the semimembranous muscle. The sample was a core approximately 2 inches in diameter and 3 inches long, and was immediately divided into representative portions for moisture, fat, ash, collagen analysis, and shear tests. The moisture, fat, and ash were determined by standard methods and the collagen determined by the method of Hartley and Hall (1949). The shear test was made on a modified core of the fresh tissue. Another small sample was fixed for histological study. Similar samples were removed from the opposite round after the feeding period at the time the cows were slaughtered. The data were treated statistically.

Results

The average daily gain for the cattle in the first experiment was 2.67 pounds and ranged from 1.34 to 4.21 pounds. With the second experiment the average daily gain was considerably lower due in part to lower feed consumption.[3] During this period grain allowances were cut down for the other cattle. However feed consumption was generally lower than in the first experiment. The average daily gain was 1.88 pounds, and ranged from a low of 1.50 to 2.12 pounds. All cattle increased one grade except one cow in the first experiment that was in the same grade at the end of the feeding period.

In table 1 the average values are given for the muscle constituents for the two experiments.

From these averages one observes that the amount of collagen present decreased with a general increase in fat deposited in the muscle. The decrease in moisture was obtained due to the increase in fat. The amount of ash did not change due to fattening. Since the cows were on feed for only 70 days and the cows were relatively old, no difference in ash content was expected. Statistical analysis of the data is given in table 2.

As indicated in table 2, there was a highly significant decrease in collagenic nitrogen during the feeding period. However when analyzed statistically on a moisture-free, fat-free basis for the two experiments, there was no significant difference; yet there was a significant difference between experiments. The results of the first experiment were highly significant between treatments, indicating that the amount of collagen

[3] At the end of 4 weeks one cow was dropped from the experiment because of acute mastitis.

TABLE 1. MUSCLE CONSTITUENTS

Sample	% Collagen (total nitrogen basis)	% Fat	% Moisture	% Ash
Experiment I (Prior to feeding period)	2.06	3.04	76.13	1.08
Experiment I (After feeding period)	1.45	7.59	74.59	1.09
Experiment II (Prior to feeding period)	2.49	4.63	73.59	1.06
Experiment II (After feeding period)	2.24	8.21	73.35	1.05

was reduced during the fattening period; whereas the second experiment results only indicated a trend in the same direction which may be explained in part because the degree of fattening of individuals was not the same.

Since there was no significant interaction for percent collagen between experiments and treatments when analyzed on a total nitrogen basis, there was no differential response; therefore the differences must be due to the same factors. Or, since all cows responded the same the differences must be due to the changes brought about by the fattening process. There was a highly significant increase in fat. However the ash and water content did not change sufficiently to show a difference when analyzed statistically, although a general decrease in moisture was noticed. There was a general increase in total protein found in the muscle at the end of the feeding period.

Shear tests on the raw samples showed a highly significant tenderizing

TABLE 2. ANALYSIS OF VARIANCES

Source of variation	Degrees freedom	% Collagen (total nitrogen basis)	% Collagen (moisture-free, fat-free basis)	% Fat	Shear tests
Treatments	1	26.97*	0.17	106.48*	107.92*
Experiments	1	110.09*	197.08*	5.20	95.53*
Experiments x treatments	1	5.24	7.38	0.42	153.34*
Animals within experiments	13	4.60	4.68	7.54	18.45

* $P<0.01$.

effect due to fattening in the first experiment but not in the second. By nature of the sample taken for shear tests, a modified core was developed. The sampling with this modified core may not have been as representative as the cylindrical standard core and may explain in part the highly significant variation found between years in the treatments effect.

Histological examinations of the muscle tissue seemed to indicate more finely divided connective tissue fibers after feeding as compared to larger connective tissue bundles in the muscles prior to feeding.

Summary

Two experiments with 8 cutter cows fed for a period of 70 days, which brought them to the utility grade, were made to determine the effect of fattening on certain physical and chemical changes in the semimembranous muscle. At the end of the feeding period the increased fat content of the muscle proved to be highly significant and so likewise the tenderness shear values. There was a highly significant decrease in collagenic nitrogen calculated as percent of total protein nitrogen; yet this difference was not significant on a moisture fat-free basis.

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FURTHER DETERMINATIONS OF THE NUTRITIVE VALUES OF FORAGES[1]

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THE increased practical and scientific interest in grassland agriculture during the past decade has been exemplified by grassland conferences in this country and abroad including several of international scope. Grass, in the sense of grassland agriculture, includes not only the grasses but also the various members of the legume family. The realization that good management of grassland is the key to livestock production, soil conservation, soil productivity and permanency of agriculture has resulted in widespread interest in the relative nutritive value of common forages, methods of preservation of forages, and managerial practices. The use of forages as a major source of essential food nutrients has thus become a subject of keen interest.

A part of the work to be reported in this paper constitutes an extension of the earlier cooperative project[2] in which the relative nutritive values of alfalfa and several grasses were determined by digestibility trials and calorimetric experimentation with sheep (Swift *et al.* 1950), the forages being harvested at comparable stages of maturity and subjected to the same experimental treatment. Since the duplicate determinations of metabolizable energy of Kentucky bluegrass were not satisfactorily concordant in the work cited above, the nutritive value of this grass was determined again in the present study. Ladino clover was also included.

Another objective of the present investigation was to compare successive cuttings of orchard grass and of brome grass throughout the entire growing season, these cuttings being made when the forage had reached the stage of "good pasturage". Six such cuttings of orchard grass were obtained (table 1) from May 15 to October 10, whereas the limited growth and disease of the brome grass permitted only two cuttings. The last sample of orchard grass, harvested October 10, had been subjected to two severe frosts.

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In addition to the studies of the nutritive value of the various forages used as pasture plants, three cuttings of timothy were harvested for hay. The three cuttings were made, "too early", "on time", and "too late". The "too early" cutting (June 6) was made when the head had emerged from the boot but before flowering. The "on time" cutting was made during the early bloom stage; the "too late" cutting just past full bloom. The same forage plots were used as are described in the paper cited above except that the ladino clover was obtained from a different experimental plot. Within a half hour after cutting, all forages were placed in a steam heated drier at a temperature of 180° F. with forced ventilation. The time required for drying was about 5 hours.

Experimental Procedure

Similar to the previous work on this subject (Swift *et al.* 1950), the method of the nutritive evaluation of the forages consisted of digestion and calorimetric experiments with sheep in which a given forage constituted the sole feed. Sixteen uniform Hampshire wether lambs averaging 83 pounds in weight were used in the experiment. After being drenched with phenothiazine they were shorn and divided into four groups of four each. Each collection period was preceded by a period of 14 days, the first seven of which constituted a transition period during which the sheep gradually became accustomed to the change in feed. The daily feed intake was kept constant during the following week (preliminary period) and during the collection period of 11 days and the subsequent calorimeter period of two days. The sheep were given equal amounts of feed each morning and evening, all feed portions for an entire period being weighed to the nearest gram at the start of the preliminary period. Samples for analysis were taken when the individual feeds were weighed. Very satisfactory separate collection of feces and urine was made with the metabolism crates described by Bratzler (1951). Feces from each sheep were collected daily, dried in a forced draft oven at 55° C., air-dried, weighed and ground in a Wiley mill in preparation for analysis. The daily urine from each sheep was made up to volume and aliquoted. A composite 11-day sample was secured from each individual and preserved with sulphuric acid. Determinations of methane, carbon dioxide and heat production were made in the respiration calorimeter. Details of procedure have been given elsewhere (Swift *et al.* 1948, Swift *et al.* 1950). The dates of harvesting and the schedule of experimentation are shown in table 1.

TABLE 1. SCHEDULE OF EXPERIMENTATION

Period No.	Sheep group	Forage				Collection period		Calorimeter period	
		Species	Date of harvest	Amount fed daily per sheep gm.	No. of sheep used	Start	End	Start	End
1	A	Orchard grass	May 15	800	4	Oct. 13	Oct. 24		
2	B	Orchard grass	May 26	800	4	Oct. 13	Oct. 24		
3	C	Brome grass	May 16	800	4	Oct. 20	Oct. 31		
4	D	Timothy	June 6	800	4	Oct. 27	Nov. 7	Nov. 8	Nov. 10
5	A	Orchard grass	June 23	800	4	Nov. 10	Nov. 21		
6	B	Orchard grass	July 20	800	4	Nov. 10	Nov. 21		
7	C	Brome grass	May 29	800	4	Nov. 17	Nov. 28		
8	D	Timothy	June 20	700	4	Nov. 24	Dec. 5	Dec. 6	Dec. 8
9	A	Orchard grass	Aug. 24	800	4	Dec. 8	Dec. 19		
10	B	Orchard grass	Oct. 10	800	3	Dec. 8	Dec. 19	Dec. 20	Dec. 22
11	A	Timothy	June 30	700	2	Dec. 28	Jan. 8	Jan. 9	Jan. 11
12	D	K. bluegrass	May 25	800	4	Jan. 5	Jan. 16	Jan. 17	Jan. 19
13	B	Ladino clover	July 19	800	4	Jan. 19	Jan. 30	Jan. 31	Feb. 2

In evaluating the three samples of timothy hay harvested at different stages of maturity, but otherwise treated alike, it was intended that the same group of four sheep (group D) be used in all three trials. However, after completing the two periods involving the "too early" and "on time" cuttings, the sheep became unwilling to consume the "too late" cutting. It was possible to complete the test only by using another group of sheep (Group A) though final results were obtained on only two of the four individuals of this group (Period 11, table 1). This hay was coarse, woody and obviously not relished by the sheep.

In period 10 only 3 sheep were used because of the limited amount of orchard grass forage available from this cutting. Four sheep were used in each of the other 11 periods no substitutions being necessary, that is, a given group was composed of the same individuals throughout the experiment.

The determinations of the nutritive values of the samples of Kentucky bluegrass and of ladino clover were made as supplementary to the earlier report (Swift *et al.* 1950) in which ladino clover was not included. Kentucky bluegrass was included in the present study because the duplicate determinations of metabolizable energy of this forage had not been satisfactorily concordant (2822 Cal. and 2427 Cal. per Kg. dry matter).

Experimental Results

Kentucky Bluegrass and Ladino Clover

The composition of the Kentucky bluegrass harvested May 25 as shown in table 2, as well as the digestibility of its constituents (table 3), is closely similar to the results obtained on comparable samples the previous year from the same plots. The metabolizable energy value (table 4) falls between the two values reported last year. Ladino clover takes a place close to orchard grass and brome grass. With the addition of ladino clover to the group of five forages on which comparative nutritive values were previously determined and with verification of the high position held by Kentucky bluegrass, it is now possible from the results of two years of work to list all six forages as used for pasture in ascending order of metabolizable energy per kilogram dry matter as follows: timothy, 2162 Cal.; alfalfa, 2290 Cal.; brome grass, 2516 Cal.; ladino clover, 2528 Cal.; orchard grass, 2537 Cal.; Kentucky bluegrass, 2647 Cal. The value for Kentucky bluegrass is the true average of both year's work from 11 sheep. These values were obtained under the strictest practicable control of experimental conditions.

TABLE 2. COMPOSITION OF FORAGES

Species	Cutting	Period No.	Dry matter %	Composition of dry matter					
				Crude protein %	Ether extract %	Ash %	Crude fiber %	N-free extract %	Energy Cal./Kg.
K. bluegrass	..	12	92.39	17.86	3.70	6.09	26.53	45.81	4596
Ladino clover	..	13	90.93	22.89	3.21	11.12	18.86	43.92	4372
Orchard grass	1	1	89.82	25.82	5.76	9.23	20.80	38.39	4695
Orchard grass	2	2	91.03	18.88	4.45	8.84	27.20	40.62	4522
Orchard grass	3	5	92.34	13.06	4.89	10.37	29.63	42.04	4427
Orchard grass[1]	4	6	91.85	20.96	5.36	8.88	28.23	36.57	4694
Orchard grass	5	9	92.09	13.23	5.99	9.31	28.23	43.23	4538
Orchard grass	6	10	92.17	13.70	5.60	10.25	26.82	43.63	4485
Brome grass	1	3	91.14	24.01	4.96	7.67	21.30	42.07	4640
Brome grass	2	7	92.29	18.29	3.61	8.54	26.72	42.84	4509
Timothy hay	1	4	91.11	24.84	3.80	6.70	31.76	32.91	4519
Timothy hay	2	8	92.13	8.35	2.69	4.70	36.29	47.98	4563
Timothy hay	3	11	92.53	6.35	1.99	4.20	38.25	49.21	4530

[1] Application of nitrogen to plots after 3rd cutting.

TABLE 3. DIGESTIBILITY OF FORAGE CONSTITUENTS

Forage	Cutting	Individual trials	Dry matter %	Crude protein %	Ether extract %	Crude fiber %	N-free extract %	Energy %
K. bluegrass	..	4	72.2	73.1	53.9	76.6	73.0	69.4
Ladino clover	..	4	75.5	76.9	44.6	70.2	83.1	72.8
Orchard grass	1	4	77.4	79.9	58.3	85.1	80.6	76.0
Orchard grass	2	4	74.0	75.1	54.6	81.5	76.4	72.2
Orchard grass	3	4	66.2	66.2	39.8	74.9	67.7	62.8
Orchard grass[1]	4	4	69.7	75.8	37.6	79.2	68.3	66.7
Orchard grass	5	4	62.9	63.9	32.1	72.8	66.1	59.0
Orchard grass	6	3	62.6	65.2	44.8	70.1	64.9	59.9
Brome grass	1	4	77.5	76.9	59.3	85.6	81.7	75.7
Brome grass	2	4	73.8	72.5	49.2	81.8	76.1	71.5
Timothy hay	1	4	67.4	82.4	57.0	72.2	56.7	64.8
Timothy hay	2	4	58.8	51.8	46.6	60.5	61.3	56.0
Timothy hay	3	2	50.0	34.6	31.0	51.5	54.1	47.3

[1] Application of nitrogen to plots after 3rd cutting.

Orchard Grass and Brome Grass

The protein content of the first three successive cuttings of orchard grass decreased as the season progressed (table 2). The two cuttings of brome grass though only 13 days apart likewise show a considerable

TABLE 4. METABOLIZABLE ENERGY, DIGESTIBLE ENERGY, DIGESTIBLE DRY MATTER AND TOTAL DIGESTIBLE NUTRIENTS

Forage	Cutting	Per kilogram dry matter			
		Metabolizable energy Cal.	Digestible energy Cal.	Digestible dry matter kg.	Total digestible nutrients kg.
K. bluegrass	..	2594	3190	.722	.713
Ladino clover	..	2528	3179	.755	.705
Orchard grass	1	....	3570	.774	.768
Orchard grass	2	....	3266	.740	.728
Orchard grass	3	....	2780	.662	.637
Orchard grass[1]	4	....	3132	.697	.678
Orchard grass	5	....	2678	.629	.619
Orchard grass	6	2128	2684	.625	.617
Brome grass	1	....	3513	.775	.776
Brome grass	2	....	3221	.738	.717
Timothy hay	1	2412	2926	.674	.669
Timothy hay	2	2120	2556	.588	.585
Timothy hay	3	1716	2144	.500	.499

[1] Application of nitrogen to plots after 3rd cutting.

difference in content of protein, ether extract, and crude fiber. Immediately after the third cutting of orchard grass, ammonium sulfate (40 lb. nitrogen per acre) was applied to the plots, which resulted in a definite increase in the protein content as found in the fourth cutting. It is to be emphasized that the samples of orchard grass and brome grass represent growth taking place since the time of the previous cutting. Each plot was cut at the date given in table 1.

A general tendency toward lower digestibility as the season advanced is shown in table 3. However, the effect of the application of nitrogen to the orchard grass plots following the third cutting, previously referred to as increasing the protein content of the forage, is also seen in the increase in digestibility of most of the feed constituents as determined

on the next cutting. The cutting of brome grass obtained May 16 was, on the basis of apparent digestibility (table 3), superior to the one obtained May 29, both being superior to the one of the previous year harvested June 1. The difference may be due to the greater degree of maturity of the brome grass in the first year's report, although it is understood of course that a given forage will not arrive at a particular stage of growth at a given date, climatic conditions of each season being effective in determining this factor. With the exception of the last

TABLE 5. NUTRITIVE YIELD OF FORAGES

Forage	Cutting	Dry matter per acre Tons	T. D. N. per acre Tons	Digestible energy per acre Therms.
Orchard grass	1	.604	0.46	1956
Orchard grass	2	.707	0.51	2095
Orchard grass	3	.340	0.22	857
Orchard grass[1]	4	.439	0.33	1247
Orchard grass	5	.221	0.14	537
Orchard grass	6	.120	0.07	292
Total per season	..	2.431	1.70	6984
Brome grass	1	.633	0.49	2018
Brome grass	2	.643	0.46	1879
Total per season	..	1.276	0.95	3897
Timothy hay	1	1.07	0.72	2840
Timothy hay	2	3.64	2.13	8440
Timothy hay	3	(3.64)[2]	(1.82)[2]	(7080)[2]

[1] Application of nitrogen to plots after 3rd cutting.
[2] Assumed.

cutting of orchard grass, metabolizable energy values of these cuttings of brome grass and orchard grass were not determined, as this had been done the previous year (Swift *et al.* 1950). Metabolizable energy was determined on the last cutting of orchard grass to learn what effect frost might have on its nutritive value.

The data in table 4 show the increase in feed nutrients in the orchard grass brought about by the application of nitrogen. There is otherwise a definite tendency toward a steady decline in nutritive value of the forage as the season progresses. There is no evidence that frost reduced the nutritive value of the last cutting. The low metabolizable energy value of the last cutting (2128 Cal.) as compared to the one established

during the previous year on earlier cuttings (2537 Cal.) is evidently a result of this progressive drop and not ascribable to frost for there is no significant difference between the 5th and 6th cuttings (table 4) with regard to either digestible energy, digestible dry matter, or total digestible nutrients.

Yield data were obtained from the experimental forage plots and it is of considerable interest to compare the season's yield of various forages from the standpoint of nutritive value (table 5). The yield per acre was determined by measuring accurately the yield on several small areas (10 x 3 feet), and raising this value to an acre basis. It is recognized that this procedure may result in magnification of experimental error but it is also reasonable to assume that large differences may be confidently considered to be significant. The nutritive values of the cuttings of orchard grass and brome grass, expressed as total digestible nutrients and as digestible energy, are listed in table 5. The contribution of orchard grass for the season is definitely superior to that of brome grass. The values for T.D.N. and digestible energy run parallel to each other throughout the series of measurements. This is not surprising as each is a feed-minus-feces value. It is reasonable to expect close agreement between these values determined on any feed. The superiority of the determination of digestible energy over that of total digestible nutrients, from the standpoint of time and experimental error involved, has been previously pointed out (Swift *et al.* 1950). Lofgreen (1951) has also shown the desirability for the use of digestible energy as a simple direct method of obtaining the equivalent of total digestible nutrients. It is to be kept in mind of course that both methods ignore the losses in the urine and methane while the determination of metabolizable energy takes these losses into account.

Timothy Hay

Attention is called to the prominent drop in protein content of timothy hay as the harvest date advanced (table 2). A decrease is also noted in the ether extract and ash with a rise in crude fiber and N-free extract. The outstanding drop in the digestibility of all feed constituents of timothy hay except the nitrogen-free extract as the harvest date becomes later, is evident from the values shown in table 3. The early cutting of timothy hay with its higher protein content and higher digestibility furnished 5.3 times as much digestible protein per ton as did the late cutting.

The decrease in the nutritive value of the hay on a weight basis

concomitant with a greater degree of maturity of the timothy (table 4) is too obvious to need special comment. Opposed to the decrease in nutritive value is the increase in yield as the harvest date is made later. Quality and quantity have been combined in table 5 to express the yield per acre of the early and on time harvests in terms of total digestible nutrients and digestible energy. Reliable yield data were not obtained on the too late harvest but on the assumption that the weight of this harvest was the same as the on time value, the nutritive value of the late cutting was at least 15 percent less than that of the on time harvest. The decrease in metabolizable energy per kilogram dry matter of the late cutting is 19.1 percent (table 4), while decreases in total digestible nutrients and digestible energy (table 5) are 14.7 percent and 16.1 percent respectively. These findings are in accord with comparable work reported by Staples *et al.* (1951) in which cattle and sheep were used in digestion, live weight, and yield studies.

Summary

In a series of 49 digestion and calorimetric experiments with sheep, nutritive values were determined on (1) Kentucky bluegrass and ladino clover harvested to simulate grazing, (2) on successive cuttings of brome grass and of orchard grass throughout the entire growing season, and (3) on timothy hay harvested, (a) before flowering, (b) at the early bloom stage and (c) past full bloom.

From the results of the present study combined with those of earlier work, Kentucky bluegrass takes first position of six common forages tested, whether the criterion be metabolizable energy, digestible energy, or total digestible nutrients. The metabolizable energy value of ladino clover is essentially identical to that of brome grass and orchard grass. The season's yield of orchard grass is greater than that of brome grass. The nutritive value of orchard grass decreases as the season progresses but is definitely improved as to composition and digestibility by the application of nitrogen in mid-season The nutritive value of timothy hay per acre may be reduced 15 percent by a delay in time of harvest.

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THE COMPARATIVE NUTRIENT CONTENT OF FIELD-CURED AND BARN-CURED ALFALFA HAY[1]

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THE proper preservation of forage crops for the feeding of livestock when pasture is no longer available is a continuing problem in animal husbandry. The preparation of silage and of hay represent the two main methods of preserving the nutrients of farm crops for winter use.

The goal in hay making is to preserve the nutrients in the crop, particularly the least stable nutrients, the vitamins. The chief causes of wastage of nutrients during field curing are the loss of leaves by shattering, the leaching of nutrients by rain, and the chemical changes occurring in the plant tissues, due to enzymic action, oxidation and photolysis (Bernstein and Thompson, 1947).

The importance of preventing the loss of leaves in hay making is well described in the following quotations from Ames and Boltz (1912):

"The leaves are the most important part of the alfalfa hay crop, since they contain the greater part of the mineral elements, as well as the bulk of the nutritive constituents for which the alfalfa plant is so highly valued for feeding purposes.

"Considerable care should be exercised in harvesting this crop, since under the most favorable circumstances the loss of leaves may amount to about 15 percent of the crop."

According to this Ohio work, the leaves make up 60 percent of the weight of the alfalfa plant when harvested for hay and contain approximately 80 percent of the protein and, except for potassium, the greater portions of the mineral elements that have been assimilated by the plant during the growing period.

The barn curing of hay has been developed to remove some of the uncertainties of field curing. By putting forage crops in the mow in the

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wilted instead of the dry state, leaf shattering is minimized, and by drying under cover the weather hazard is reduced, or entirely avoided if heated air, rather than unheated air, is blown through the air ducts in the mow (Jennings, 1945). The acquisition of vitamin D potency by solar irradiation during the wilting of hay prior to curing in the barn may be sufficient to prevent rickets in growing calves if fed at the rate of 1.2 to 1.7 pounds per 100 pounds body weight (Moore *et al.*, 1948).

Weaver and Wylie (1939) present a good review of the development of barn-drying methods, together with a summary of five years' work at the University of Tennessee. This experience leads them to believe that under Tennessee conditions, "Hay dried in the barn is one grade and class better, according to analysis, than the same hay dried in the field. The barn-dried hay averages 2.5 percent more leaves and 19 percent more green color." It was generally considerably richer in carotene and higher in protein on the dry basis, though there were no consistent differences in ash, crude fat, crude fiber or nitrogen-free extract. Essentially the same comparative ratings of field- and barn-cured hay were reported by Hudson (1941). However, Archibald and others (1951) were unable to detect any essential differences in the composition of field-cured and barn-cured hays at the termination of a normal storage period, although "Hays in which the curing process was completed by mow drying were significantly higher in carotene at time of storage than those completely cured in the field." Differences between hays cured by the two processes may be entirely obscured unless the botanical composition of otherwise comparable samples is the same.

Some feeding trials with field-cured and barn-cured hay have favored the latter distinctly (Wylie, 1939), or moderately (Lutz and Wolcott, 1949), but some investigations have not detected significant differences (Keener *et al.*, 1949; King *et al.*, 1950; Archibald *et al.*, 1951). The usually large experimental error in animal feeding trials may obscure even considerable differences in the nutritive value of rations if the experimental groups are small, and particularly if the experiment is not designed to test critically the comparative value of the two hays in those nutrients, studied in turn, in which differences may be presumed to exist. However, weather conditions prevailing at the time of cutting may be a determining factor in the magnitude of the differences between the products of the two curing methods.

In the belief that more information is desirable for the proper evalua-

tion of barn-curing versus field-curing of hay, particularly as regards the labile constituents of forage crops and the effect of weather conditions, the investigation to be reported below was undertaken.

Materials and Methods

This investigation involved the analysis of samples of alfalfa of the same stand, part of which was field-cured, the rest having been barn-cured. These samples were collected on farms in northern Illinois operating barn-curing equipment. In order to understand the changes in the chemical composition of the forage throughout the period of wilting, field-curing or barn-curing, respectively, and subsequent storage, the samples were hand-separated in the laboratory into their leaf and stem fractions, which were weighed and analyzed separately for carotene, riboflavin, dry matter, nitrogen, ether extract, crude fiber, ash and gross energy.

The time of cutting the alfalfa and the length of time the cut alfalfa remained in the field before removing to the barn, for either method of curing, were determined by conditions beyond the control of the authors, particularly weather conditions and availability of labor. The alfalfa was cut in the early or late bloom stage. The weather, in so far as incomplete records indicate, was clear generally. The alfalfa to be cured in the barn remained in the field from one to two days, by the end of which time the dry matter content increased from an average of 30 percent to levels varying from 57 to 83 percent, with one sample (farm 34) containing less dry matter, 32 percent, than when cut, 35 percent, for reasons not evident from the records. Thus, these samples were dried to a greater degree in the field than need be for successful barn drying. Weaver and Wylie (1939) state, "Repeated tests proved that the barn-drying system can complete the drying of hay of 45 percent moisture. It took from 4 days to 2 weeks, depending upon weather conditions and the amount of hay stored."

Sampling and Care of Samples

Staff members of this university collected samples weighing about 4 pounds each from six fields on five farms in a manner designed to secure as representative samples as feasible. In three of these farms barn-curing was done by forcing unheated air through the mow; two farms (No's. 20 and 31) forced electrically heated air.

The alfalfa from each field was sampled before cutting (except on

farm nine), at the time of removal to the barn, at the end of the storage period of 144 to 307 days, averaging 237 days, and on farms nine and 20 at two or three intermediate times. The terminal storage samples for hay from the same field, cured in the barn or in the field, were stored for the same time. The forage was generally put in the mow in the loose chopped condition, but on farms 30 and 31 the hay cured in the field was baled for storage, and on farm 31 the forage for barn curing was also baled. No barn-cured hay was obtained from farm 20.

The samples were packed at the farms in 5-gallon cardboard containers made relatively moisture-proof and heat-insulated by a lining of several layers of paper with paper pulp between them. These samples were either immediately frozen with dry ice or quickly put in cold storage. All samples were transferred to deep freeze (—15° C.) storage bins at the University, where they remained until analyzed.

Analytical Methods

Leafiness. Hand-separation and weighing of leaves (including blossoms), stems, and non-alfalfa material, were done on high-moisture samples as soon as possible at freezing temperature and in dim light. All non-alfalfa material was discarded immediately. The leaf and stem fractions thus obtained were stored in dark glass jars at —15° C. until analyzed.

Low-moisture samples were treated in the same manner except for the fact that hand-separation was performed in a cool room.

Carotene. Relatively large duplicate samples of the leaf fraction (15 to 20 gm.) were taken for this determination. The method of carotene determination by phasic separation outlined by Johnson (1948) was adopted and, after proper checking, applied with some modification. The essential modification consisted in the homogenizing of all samples (dry or fresh green) in the Waring Blendor with hot alcoholic KOH prior to refluxing in the same solution.

The pigments in alfalfa determined by this method seem to consist essentially, to the extent of about 95 percent, of β-carotene, judging from the observations of Derby and De Witt (1948). However, the modified A.O.A.C. method, when applied to alfalfa, gave a carotene value 1.36 times that obtained with the alcoholic KOH method used in this investigation. The difference is due, mainly, to a more efficacious extraction of β-carotene.

Dry Matter. Simultaneously with the taking of samples for carotene determination, 150 to 200 gm. samples of the leaf and stem fractions

were dried to constant weight in a vacuum oven at 65 to 70° C. The dry material was rapidly ground in a small Wiley mill, put through a 20-mesh sieve, and stored in tightly stoppered glass jars for further determination of nitrogen, ether extract, crude fiber, ash, and gross energy.

Riboflavin. Relatively large samples (5 to 7 gm.) of the leaf and stem fractions were homogenized for ten minutes in a Waring Blendor with 200 ml. of 0.1 N HCl. Afterwards the *Lactobacillus casei* assay method for riboflavin was applied as outlined by Johnson (1948).

Nitrogen, Ether Extract, Crude Fiber, and Ash were determined according to current A.O.A.C. methods with slight modifications. The gross energy of the samples was measured with the Parr oxygen-bomb adiabatic calorimeter.

Results

The alfalfa crop at time of cutting, from the five fields from which such samples were secured, possessed the average composition summarized in table 1 (averages and standard errors). The leafiness, determined by hand separation averaged 48.5±2.5 percent on the dry basis, and the leaves contained preponderant proportions of carotene, 82 percent, riboflavin, 75 percent, and protein, 69 percent, in the entire crop. Each mean value cited below will be accompanied by its standard error.

Time Changes in the Composition of Alfalfa During Curing and Storage. Figures 1 to 4 reveal graphically the average changes that occurred in the moisture and carotene content of the forage prepared from the alfalfa crops, based upon pooled data from all farms. Practically all of the drying of the forage in both field-curing and barn-curing methods took place within the first ten days following cutting. Therefore, this period may be considered the curing period. The storage period is the time from ten days to final sampling 144 to 307 days from cutting.

Figure 2 shows a plot of the moisture percentages during the storage period for both curing methods; straight lines were fitted to these points by the method of least squares. The coefficient of regression of both lines did not differ from zero significantly, so the lines were drawn with zero slope. The mean percentages of moisture of field-cured and barn-cured hays were 12.0±1.2 and 11.1±0.9, respectively. These values are statistically indistinguishable, as expected.

Since no statistically significant changes in moisture contents were detected beyond the curing period, segments of a hyperbola were fitted

TABLE 1. THE AVERAGE COMPOSITION OF THE ALFALFA AT CUTTING: FIVE SAMPLES

All values are expressed on the dry basis:[1] means with standard errors

	Leafiness	Carotene	Ribo-flavin	Crude protein	Crude fiber	Ether extract	Ash	Gross energy
		p.p.m.	p.p.m.	%	%	%	%	Cals./gm.
Leaves	.....	276±45	35.4±2.3	27.2±0.5	13.9±0.4	1.97±0.28	8.45±0.03	4.57±0.16
Stems	.....	56±9	11.0±0.8	11.7±0.2	42.5±0.8	1.14±0.06	4.82±0.53	4.40±0.05
Total crop	48.5±2.5	163	22.8	19.2	28.6	1.54	6.58	4.48
% in leaves	.....	82	75	69	24	62	62	49

[1] Dry matter in leaves=30.1±1.7
Dry matter in stems=29.4±1.6

by the method of least squares to the points plotted in figure 1 and to the weighted mean of the corresponding points during the storage period shown in figure 2, i.e., 12.0 x 6 and 11.1 x 8, respectively. An arbitrary time origin of 0.1 day was adopted for the fitting of these hyperbolas because the logarithm of zero is minus infinity. Examination of these points and curves reveals there was little difference between

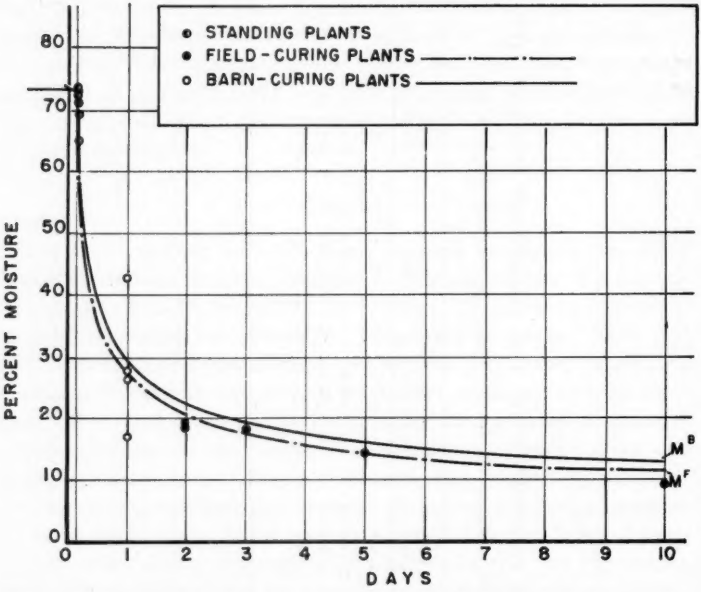

Figure 1. Changes in moisture content of the whole plant during the curing period by the field-curing (M^F) and the barn-curing (M^B) methods.

the rates of dehydration in the field-curing and barn-curing methods, probably because the alfalfa to be barn-cured was left in the field for a longer period than necessary, as mentioned above.

In both field-curing and barn-curing methods, leafiness during the curing period was found to be statistically indistinguishable from leafiness during the storage period. The average percentage leafiness at cutting time was 48.5 ± 2.5 on the dry basis; throughout the curing and storage periods, the average percentage leafiness was 33.0 ± 1.6 for the field-curing method, and 39.6 ± 1.4 for the barn-curing method. The following differences are significant at the indicated levels: $48.5 - 33.0 = 15.5$ ($P < 0.001$), and $48.5 - 39.6 = 8.9$ ($P < 0.005$). These P

values correspond to one-tailed t-tests, since there are sound reasons for expecting some leaf shattering after cutting, no matter which curing method is used.

Figure 3 shows the changes in carotene contents of the leaf and stem fractions during the curing period for each curing method. Figure 4 shows a plot of the carotene contents of these fractions during the storage period. Straight lines were fitted to these four sets of data by

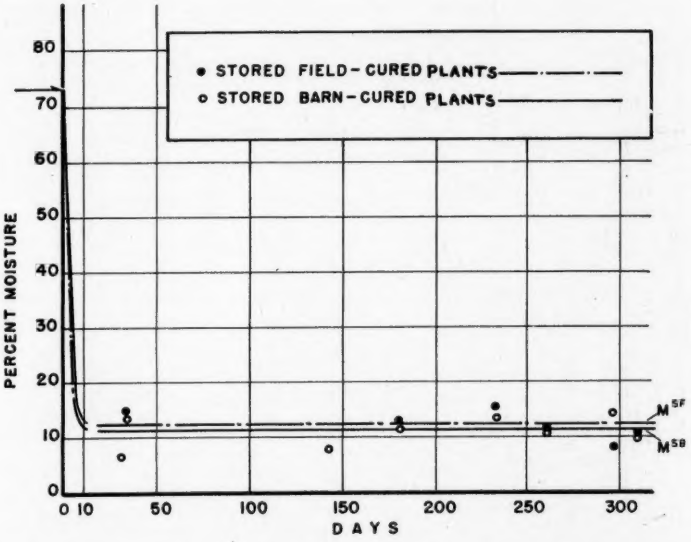

Figure 2. Moisture content of the whole plant during the storage period by the field-curing (M^{SF}) and the barn-curing (M^{SB}) methods.

the method of least squares. The coefficient of regression of each of these lines did not differ from zero significantly; therefore, the lines were drawn with zero slope. It appears that the variability of commercial alfalfa hay is too large for the detection, from population samples of the size used here, of any gradual loss of carotene that may have occurred on storage.

By the same reasoning as in the case of moisture percentages, segments of hyperbolas were fitted to the points plotted in figure 3 and to the weighted mean of the corresponding points during the storage period. The fitting was done by the method of least squares.

Figures 3 and 4 show how close to each other these curves and lines

run. The destruction of carotene appeared associated with the process of dehydration, as reported before. This destruction of carotene in alfalfa, and in other plant tissues (Bernstein and Thompson, 1947), is due mainly to oxidation by light and by enzyme action.

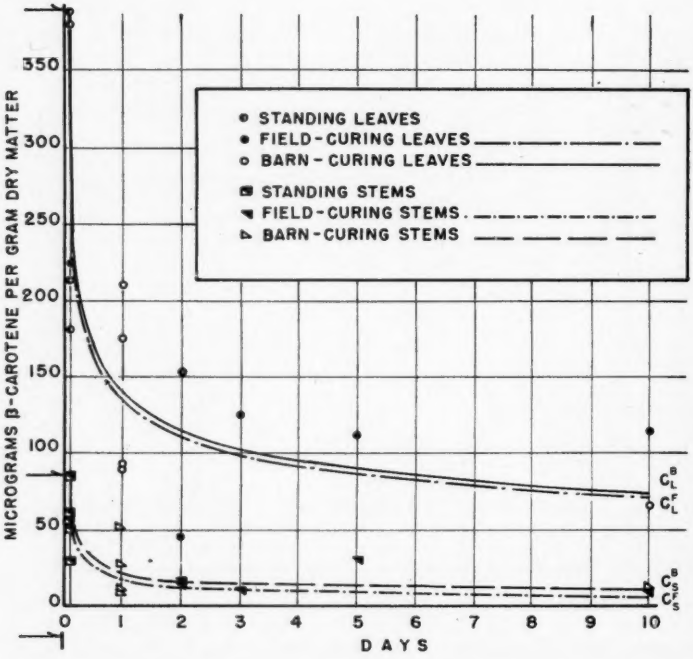

Figure 3. Changes in β-carotene content of the leaves and stems during the curing period by the field-curing (C_L^F and C_S^F, respectively) and the barn-curing (C_L^B and C_S^B, respectively) methods.

At cutting time the mean content of carotene was 276±45 p.p.m. for the leaf fraction and 56±9 p.p.m. for the stem fraction. The ratio of $\frac{276}{56}$=4.9 agrees well with previous findings (Seshan and Sen, 1942) on the distribution of carotene in alfalfa. By the end of the curing period for both curing methods, the carotene content of the leaf and stem fractions were reduced to nearly one-fourth and one-sixth of their respective values at cutting time.

Analyses of the pooled data for the purpose of detecting other changes in composition of alfalfa during the curing period revealed significant losses of riboflavin and doubtful losses of protein. However, in the field-curing method there was a significant loss of protein on the

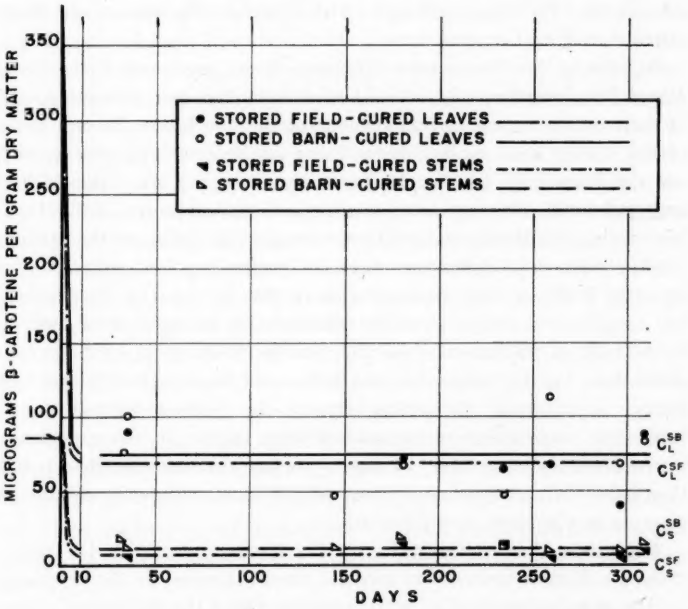

Figure 4. β-carotene contents of leaves and stems during the storage period by the field-curing (C_L^{SF} and C_S^{SF}, respectively) and the barn-curing (C_L^{SB} and C_S^{SB}, respectively) methods.

dry basis from the time of cutting to the end of the storage period, at the 5 percent probability level for the leaves and at the 1 percent level for the stems. The corresponding average losses for the barn-cured leaves and stems were quite insignificant statistically ($P=0.4$ and 0.1, respectively). Slight but statistically significant losses in gross energy per gram of dry matter were evident between the average values on the standing crop and average pooled values for the curing and storage periods: for the leaves, $4.57-4.48=0.09$ ($P<0.01$), and for the stems, $4.40-4.36=0.04$ ($P<0.02$) for the barn-curing process; for the leaves,

4.57—4.44=0.13 ($P<0.002$) and for the stems, 4.40—4.37=0.03 ($P<0.05$) for the field-curing process. Changes in energy content of leaves or stems after removal to the barn could not be established statistically. Further analysis offered suggestions that during the curing and storage of hay increases in the concentration of crude fiber and of ash on the dry basis occurred. The probable significance of these increases will be discussed later.

Differences in Composition Between Barn-Cured and Field-Cured Hays. The foregoing analysis of pooled data does not take advantage of their paired nature in that on four of the five farms the two types of hay curing were applied to the same alfalfa crop and were carried out simultaneously; also on all farms the same hay was sampled and analyzed serially throughout the curing and storage period. Full advantage of this situation is realized by the analysis according to the Student (1925) method of differences between properly paired samples. For example, if the carotene concentration on the dry basis of the terminal leaf samples is deducted from the concentration at the time of removal to the barn of the same forage, the average decreases in carotene concentrations for the barn-cured and field-cured leaves are 63.2 and 55.3 p.p.m., respectively; the probability by the Student method that a fortuitous combination of the uncontrolled factors in the experiment would bring about as large, or larger, average decreases as these is less than 0.02. The carotene in the stems did not show statistically significant changes in the same time interval.

The crude fiber concentration in the leaves, but not in the stems, showed a distinct tendency to increase when expressed on the dry basis, by the same comparison of serial samples. Since the method of curing exerted no appreciable effect on this change, all serial sample differences were pooled to give an average increase of 1.51 percentage units, significantly different from zero at the 4.4 percent level. This might be interpreted as a result of the loss of nitrogen-free extract by fermentation processes were it not for the fact that the ash percentage in the leaves shows no parallel change. The crude fiber increase may therefore be due to an increasing insolubility of its constituents in dilute acid and alkali during curing and storage.

In evaluating the final products of the barn-curing and field-curing process, advantage was taken of the fact that, except for carotene, little consistent change occurred in chemical composition on the dry basis during curing and storage. In order to minimize the sampling error, therefore, the compositions of each sample, as determined from the time of removal to the barn to the end of the storage period, were averaged,

except for the carotene values which relate in all cases but one to the final sampling. In this one case (farm 30, field 2), the results secured with field-cured samples taken at the time of removal to the barn and after 233 days of storage, suggest strongly either a confusion of sample numbers or an abnormal situation that caused the moisture content and the carotene content to increase on storage. Hence, for both field-cured and barn-cured hays from this field, the carotene values given in the tables are the averages of samples taken at the time of mowing and 233 days later. The values given in tables 2, 3 and 4 are averages of two determinations for all constituents but carotene, and averages of three determinations for farm nine, for which three samples were taken.

Tables 2, 3 and 4 were constructed to detect any differences in composition between barn-cured and field-cured hays. In the statistical analysis of the data, given at the bottom of each table, the Student (1925) method of analyzing differences between paired observations was utilized, the paired observations consisting of the two types of hay produced from the same alfalfa field.

Table 2 relates to the total hay samples, estimated from the relative dry weights of leaf and stem portions and the analytical data from each portion. From the analysis at the bottom of the table it is evident that the barn-cured hays were definitely superior to the field-cured hays in leafiness and in content of carotene, riboflavin and protein on the dry basis. They were definitely less fibrous and were indistinguishable from the field-cured hays in ether extract and in content of gross energy. Some probability of a greater ash content was established, with $P=0.067$.

Tables 3 and 4, relating to the composition of leaves and stems, respectively, cured by the two processes, show that the superiority of barn-curing over field-curing relates mainly to a greater leafiness of the barn-cured product. However, the barn-cured leaves were definitely ($P=0.0065$) richer in riboflavin than the field-cured leaves, and the barn-cured stems were definitely ($P=0.010$) richer in carotene. The barn-cured stems were also definitely lower ($P=0.043$) in crude fiber than the field-cured stem.

Practical Consideration. The experimental results have been considered thus far from the standpoint of the reality, or otherwise, of the average differences in chemical composition of hay produced by field-curing and barn-curing alfalfa cut from the same fields. If the differences are real in the statistical sense, what nutritional importance do they possess for the feeding of farm animals?

TABLE 2. THE COMPOSITION OF THE EXPERIMENTAL HAYS AT THE END OF THE STORAGE PERIOD

All results are expressed on the dry matter basis

Farm	Field	Cutting	Curing method	Leafiness	β-carotene	Ribo-flavin	Crude protein	Crude fiber	Ether extract	Ash	Gross energy
				%	p.p.m.	p.p.m.	%	%	%	%	Cals./gm.
30	1	1	Barn	43.4	52.7	15.0	17.2	29.7	1.72	6.70	4.57
30	1	1	Field	41.9	28.5	12.4	16.7	31.7	1.76	6.03	4.39
30	2	3	Barn	35.3	38.7	10.5	16.3	36.7	1.43	5.79	4.44
30	2	3	Field	29.6	24.6	10.2	15.8	39.0	1.25	5.39	4.43
31	1	1	Barn	37.0	38.0	14.1	17.4	32.2	1.87	6.22	4.32
31	1	1	Field	35.0	34.4	13.9	15.9	34.6	1.54	6.03	4.31
9	1	1	Barn	39.2	35.4	13.1	17.7	34.0	1.75	7.29	4.41
9	1	1	Field	30.9	20.0	11.3	16.0	36.7	1.84	5.59	4.42
34	1	2	Barn	34.8	32.1	13.6	16.5	35.2	1.43	6.21	4.43
34	1	2	Field	28.9	28.1	11.2	13.7	36.8	1.46	6.12	4.40
20	1	2	Barn	48.2	36.0	22.1	18.7	32.1	1.44	6.89	4.49
Averages for barn-cured hays[1]				37.9	39.4	13.3	17.0	33.6	1.64	6.44	4.43
Averages for field-cured hays				33.3	27.1	11.8	15.6	35.8	1.57	5.83	4.39
				Statistical analysis of differences between paired samples							
	Mean difference			+4.68	+12.26	+1.46	+1.40	−2.20	+0.07	+0.61	+0.044
	Standard deviation			2.87	8.65	1.14	.96	.42	.18	.65	0.077
	t value			3.26	2.83	2.56	2.92	10.52	.78	1.88	1.14
	P			0.016	0.024	0.031	0.022	<.001	.24	0.067	0.16

[1] Exclusive of the hay from Farm 20

TABLE 3. THE COMPOSITION OF ALFALFA LEAVES AT THE END OF THE STORAGE PERIOD

All results are expressed on the dry matter basis

Farm	Field	Cutting	Curing method	β-carotene	Riboflavin	Crude protein	Crude fiber	Ether extract	Ash	Gross energy
				p.p.m.	p.p.m.	%	%	%	%	Cals./gm.
30	1	1	Barn	111.0	23.8	25.7	14.3	2.49	8.62	4.46
30	1	1	Field	66.0	22.3	26.0	13.4	2.67	9.08	4.43
30	2	3	Barn	70.4	21.0	25.8	16.6	2.22	8.02	4.55
30	2	3	Field	53.6	20.3	25.3	16.8	1.70	7.58	4.53
31	1	1	Barn	84.6	29.0	26.8	13.8	2.78	9.70	4.34
31	1	1	Field	85.6	28.4	25.3	13.7	2.45	10.00	4.28
9	1	1	Barn	82.8	26.2	28.0	15.4	2.71	9.12	4.49
9	1	1	Field	60.0	25.1	27.7	14.8	2.66	9.04	4.49
34	1	2	Barn	65.5	24.7	25.1	16.9	2.01	8.65	4.47
34	1	2	Field	68.6	22.8	22.6	15.2	2.19	9.00	4.46
20	1	2	Barn	59.5	37.4	27.7	16.3	1.94	9.08	4.58
Averages for barn-cured hays[1]				82.9	24.9	26.3	15.4	2.44	8.82	4.46
Averages for field-cured hay				66.8	23.8	25.4	14.8	2.33	8.94	4.44
				Statistical analysis of differences between paired samples						
			m	+16.1	+1.16	+0.90	+0.62	+.108	−0.12	−0.024
			s	19.6	.545	1.105	0.711	.312	.34	.0229
			t	1.64	4.26	1.63	1.64	.69	.70	2.10
			P	.088	.0065	.089	.088	.29	.26	.052

[1] Exclusive of the hay from Farm 20

TABLE 4. THE COMPOSITION OF ALFALFA STEMS AT THE END OF THE STORAGE PERIOD

All results are expressed on the dry matter basis

Farm	Field	Cutting	Curing method	β-carotene	Riboflavin	Crude protein	Crude fiber	Ether extract	Ash	Gross energy
				p.p.m.	p.p.m.	%	%	%	%	Cals./gm.
30	1	1	Barn	7.9	8.3	10.6	41.6	1.13	5.23	4.30
30	1	1	Field	1.4	4.8	10.0	44.9	1.11	3.83	4.35
30	2	3	Barn	20.5	5.3	11.2	47.6	1.09	4.57	4.38
30	2	3	Field	12.4	5.9	11.8	48.3	1.06	4.47	4.39
31	1	1	Barn	10.6	5.4	11.9	43.0	1.33	4.18	4.30
31	1	1	Field	6.8	6.2	10.4	45.9	1.05	3.89	4.33
9	1	1	Barn	4.7	4.6	11.1	46.0	1.13	3.90	4.37
9	1	1	Field	2.1	5.0	10.7	46.4	1.48	4.05	4.39
34	1	2	Barn	14.3	7.7	12.0	45.0	1.12	4.91	4.41
34	1	2	Field	11.9	6.5	10.1	45.6	1.17	4.95	4.38
20	1	2	Barn	14.0	7.9	10.3	46.8	0.97	4.85	4.40
Averages for barn-cured hay[1]				11.6	6.3	11.4	44.6	1.16	4.56	4.35
Averages for field-cured hay				6.9	5.7	10.6	46.2	1.17	4.24	4.37
				Statistical analysis of differences between paired samples						
			m	+4.68	N.S.[2]	+0.76	−1.58	N.S.	N.S.	−.016
			s	2.52		.98	1.40			.029
			t	3.71	(3 re-	1.55	2.26	(2 re-	(2 re-	1.08
			P	.010	versals)	.10	.043	versals)	versals)	.17

[1] Exclusive of the sample from Farm 20.
[2] Not significant.

An increase of 4.6 percent in the leafiness of alfalfa hay by barn curing preserves to a greater extent all of the nutrients, proteins, vitamins and minerals, which are prominent components of the leaf as contrasted with the stem. In terms of hay grading, it amounts to about one-third of the difference between U. S. grades No. 1 and No. 2 (U.S.D.A., 1944).

A difference in protein content of 1.4 percent on the dry basis in the hay means a corresponding saving of protein in the concentrate feed. For example, Morrison (1948) proposes for dairy cattle a series of grain mixtures containing from 12 to 24 percent of protein, depending upon whether the roughage fed is alfalfa, soybean or cowpea hay (averaging about 16 percent protein) at the one extreme, or non-legume roughage (averaging about 8 percent or somewhat less in protein) at the other extreme. A difference in protein content in the roughage of 1.4 percent on the dry basis (about 1.25 percent on the fresh basis) means a saving of 1.9 percent protein in the grain mixture, according to this scheme of feeding.

The saving of 45 percent in the carotene content of alfalfa hay associated with barn-curing is a clear nutritional advantage since green leaves are the most important source of this provitamin for all farm animals. The 12 percent increase in riboflavin may possess little significance for ruminants but considerable for non-ruminants, grains and their by-products being such poor sources. According to Jennings (1949), non-ruminants in the years 1945 to 1947, inclusive, consumed about 20 percent of all hay consumed on farms in this country. The gastro-intestinal synthesis in these animals, even in the horse (Carroll, Goss and Howell, 1949), apparently is not a considerable item in their nutrition.

A lowering of 2.2 percent in the crude fiber content of alfalfa hay on the dry basis due to greater preservation of leaves during barn curing would be associated with a saving of digestible organic matter because of the high correlation existing between these two variables. The extent of this effect may be computed for different classes of livestock from the regression equations assembled by Mitchell (1942). A decrease in the fiber content of a feed of 2.2 percent on the dry basis may be expected to be associated with the following increases in the percentage digestibility of the organic matter: for cattle 1.93; for rabbits 3.41; and for poultry 4.30. From the compilation of Schneider (1947) of digestion coefficients for the horse, it has been computed that the regression of digestibility of organic matter (y) on crude fiber content

of the feed on the dry basis (x), using only those feeds, 69 in number, for which digestibility was directly determined, is:

$$y=85.07-1.053x.$$

From the coefficient of x, it may be computed a reduction of 2.2 percent in crude fiber on the dry basis would be expected to be associated with an increase of 2.3 percent in the digestibility of the organic matter of a feed.

If the above discussed improvements in the nutritional quality of alfalfa hay associated in these experiments with barn curing cannot be demonstrated in the ordinary feeding trials with farm animals, it is more a comment on the insensitivity of such trials than on the significance of the improvements established by mechanical, chemical and microbiological analysis of the hays. There is good reason to believe that in actual practice in the feeding of hay-consuming animals, the differences established in this experiment between the two methods of curing alfalfa forage would be of economic significance in the long run.

Summary and Conclusions

For the purpose of determining whether the barn curing of alfalfa hay will yield a more nutritious product for livestock feeding than the field-curing method, alfalfa from five fields on four different farms in Illinois was cured by the two processes and sampled at different stages in the curing and storage periods. Each sample was divided manually into leaf and stem portions, and each portion was weighed and analyed for dry matter, carotene, riboflavin, protein (N x 6.25), crude fiber, ether extract and ash, and its heat of combustion (gross energy) determined in the bomb calorimeter. The analytical and thermochemical results were compared on the dry matter basis.

The measurements thus obtained and expressed were submitted to statistical analysis and justify the following conclusions:

1. Under the weather conditions that prevailed and the management practices that were followed, the rate of dehydration of the hay was much the same by the two methods of curing and was completed within a ten-day period.

2. During the initial period of about ten days there was a greater loss in leafiness in the field-cured than in the barn-cured hay.

3. The barn-cured hays were definitely superior to the field-cured hays in their contents of carotene, riboflavin and protein expressed on the dry basis, and contained definitely less crude fiber. The differences are largely, but not entirely, traceable to the greater leafiness of the

barn-cured hays, 37.9 percent on the dry basis, as compared with 33.3 percent for the field-cured hay. At cutting, the leafiness of the alfalfa, expressed on the same basis, was 48.5 percent.

4. Even under the generally fair weather prevailing during the hay curing in this investigation, the barn-curing method yielded a product containing 14 percent more leaves, 45 percent more carotene, 12 percent more riboflavin and 9 percent more crude protein than the field-curing method. Under less favorable weather conditions and a shorter period of exposure to the weather of the hay to be barn-cured, one might expect greater differences in the nutritive value of hays cured by the two processes.

5. The importance of these differences in nutritive value between the field-cured and barn-cured alfalfa hays in practical livestock feeding operations seems to be considerable.

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RATE OF VITAMIN D FORMATION IN HAY[1]

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THE main sources of vitamin D for cattle are roughages and exposure of animals to direct sunshine. Since our northern climate is composed of short summers and long winters, many calves do not receive much sun exposure until 8 or 9 months of age. Therefore, roughages, especially hays, are of prime importance in providing sufficient amounts of this vitamin for growing cattle.

Theoretically, no vitamin D is considered to be present in the green growing plant. However, as soon as it is cut and exposed to the radiant energy of the sun, the provitamins present, such as ergosterol, are changed to the vitamin. Little is known regarding the rate of this transformation. How soon after cutting the hay crop does any appreciable amount of vitamin D occur?

Previous work by Newlander (1948) showed barn-cured hay to have practically as much vitamin D as sun-cured even though left in the field for only approximately 24 hours as compared with 48 hours for the field-cured hay. This is shown in the following table.

TABLE 1. VITAMIN D IN HAY (TIMOTHY)

	Barn-cured	Field-cured
	U.S.P. units per gram	
1946 crop, cut 6/27	0.51	0.61
1947 crop, cut 7/14	2.33	2.00
1948 crop, cut 7/8	1.50	1.74

Moore *et al.* (1948), on the other hand, found but little more than half as much vitamin D in barn as in field-cured hay. However, the amount proved sufficient to prevent rickets in growing calves if the usual levels of roughage feeding were followed.

Wallis *et al.* (1949) made a nation-wide survey of the vitamin D

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content of roughages. The results revealed striking and unpredictable variations in amounts of this vitamin. They concluded that many other factors, beside extent of sun exposure in the curing process, are responsible for the amount of vitamin D in roughages.

Experimental

In order to throw further light upon this question of vitamin D formation, samples of hay crops exposed to the sun for different lengths of time were collected and assayed. A sufficient amount of the crop for several samples was cut with a scythe and left exposed to the sun for definite periods of time. The samples were then collected and the drying completed in a dark loft, except those dried in the field to hay. These dried samples were then milled and assayed. The weather was clear except as noted.

Results and Discussion

Table 2 shows the vitamin D content of the different samples:

TABLE 2. VITAMIN D IN HAY SAMPLES

Lot No.	Type of Crop	Sample collected	U.S.P. units per gram
1	Brome and Alfalfa	7 a.m. 6/28	0.11
	Cut 7 a.m. 6/28/50	7 p.m. 6/28	0.20
		12 noon 6/29	<1.00
		10 a.m. 6/30	1.60
2	Alfalfa, 2nd cutting	7 a.m. 8/15	0.07
	Cut 7 a.m. 8/15/50	11 a.m. 8/16	0.28
		4 p.m. 8/17	<2.00
3	Brome	1:30 p.m. 6/21	0.10
	Cut 1:30 p.m. 6/21/51	1:30 p.m. 6/22	0.87
		Noon 6/24	0.52[1]
4	Alfalfa	1:30 p.m. 6/21	0.25
	Cut 1:30 p.m. 6/21/51	1:30 p.m. 6/22	0.82
		Noon 6/24	0.36[1]
5	Brome	5 p.m. 7/11	0.42
	Cut 11 a.m. 7/11/51	1 p.m. 7/13	1.19
		5 p.m. 7/13	2.78[2]

[1] Started to rain 5 p.m. 6/22, sample left under cover until noon 6/23, then put out in sun until cured.

[2] Rained 7/12 but sample left outside until cured.

According to the Recommended Nutrient Allowances for Dairy Cattle (1945) a calf would not eat a sufficient amount of the hays lowest in vitamin D content to provide the required amount of vitamin D. For example, the hay in lot 4 assayed but 0.36 units per gram or 163 per pound. A 200-pound calf would need 5 pounds daily of this hay to meet the required 800 units, whereas the normal consumption of this size animal would be around 3 pounds. However, trials by Newlander (1950) have shown barn-cured hay containing only 0.35 units per gram to supply sufficient of this vitamin for growing calves even when the animals were not allowed any sun exposure.

In general, the results shown in the two tables indicate that the longer the exposure of the hay crop the more vitamin D may be expected. It would appear that hays cut in July develop more vitamin D than those cut very early or second cuttings harvested in August.

It is not clear as to why the noon samples in lots 3 and 4 showed comparatively low results, 0.52 and 0.36 respectively. Perhaps protecting the samples from the rain and then exposing the samples when the weather had cleared, not only retarded development but caused some loss.

There is much to be learned regarding vitamin D formation but a less expensive method of analyzing hays for this vitamin needs to be developed before many data can be secured.

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THE DIGESTION OF PROTEIN *IN VITRO* AS INFLUENCED BY EXTRACTS FROM SOYBEAN FORAGE[1]

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THE influence of green soybean forage upon the digestion of protein by trypsin has not been previously investigated. The effect of the raw soybean seed upon trypsin activity as affecting protein hydrolysis, however, has been studied and was first demonstrated by Ham and Sandstedt (1944) and confirmed by Bowman (1944). A trypsin inhibitor was isolated from unheated soybeans and later crystallized by Kunitz (1945, 1946) who found the inhibitor to be a globulin. The assumption was made by Borchers *et al.* (1947) that this trypsin inhibitor was identical to a chick growth depressing factor found in the dilute acid soluble fraction (pH 4.2) of raw soybeans. Klose *et al.* (1946) have observed inhibited growth in rats fed a dilute acid soluble fraction of unheated soybeans. More recently two other less active trypsin inhibitors from the soybean have been reported by Bowman (1948).

The foregoing evidence has established the existence and some of the characteristics of the antitryptic factor in the mature soybean seed. Because the soybean plant is used as a forage for livestock, an investigation of the possible existence of a trypsin inhibitor in green soybean forage as it might relate to protein digestion seemed pertinent. This paper reports the results of a study designed to determine whether or not protein digestion is retarded by a trypsin inhibitor if present in green soybean forage.

Experimental

The procedure used in measuring tryptic activity was by *in vitro* experiments. In these studies rates of protein hydrolysis under uniform conditions as influenced by the presence of soybean plant extracts were investigated.

[1] Abstract of this study was presented at the 46th annual meeting of the American Dairy Science Association, Knoxville, Tennessee, June 7, 1951.

[2] The authors wish to thank Doctors E. E. DeTurk and C. M. Woodworth of the department of agronomy, University of Illinois, and Mr. J. L. Cartter, senior agronomist, U. S. Soybean Laboratory, for providing greenhouse facilities for the growing of the soybeans that were used in this study and E. E. Hixson and George D. Rolleri for technical assistance in the conduct of the study.

[3] Department of Dairy Science.

Soybean forage of the Lincoln variety was harvested at four different stages of plant development, namely (1) prebloom, (2) very early seed forming (seeds were 1–3 mm. in length), (3) seed one-third mature size and (4) seeds one-half mature size. The period of soybean plant growth studied was approximately seven weeks.

Forage extracts. Extracts of forage in the prebloom and seeds one-half formed stages were prepared by finely grinding the entire above-ground portions of the plants. The plant juice from the ground material was expressed through heavy cloth after which it was centrifuged at 1500 R.P.M. for five minutes. The supernatant layer was then removed and retained for study.

Soybean plants in the early seed-forming stage and those with seeds in the one-third mature stage were first separated into seed and leaf-stem fractions. The leaf-stem fraction of the plants was finely ground and treated in the same manner as was described for the plants in the prebloom and seeds one-half mature stages of development.

Seed extracts. The aqueous extracts of seeds from plants in stages two and three were each made by blending in a Waring blendor 2 grams of seeds with 98 ml. of distilled water for five minutes. The mixture was then centrifuged for five minutes at 1500 R.P.M. The supernatant liquid layer was retained for study. In the same manner, an aqueous extract was made of one-third mature soybean seeds after being autoclaved for 30 minutes at 15 pounds pressure at 120° C. This extract was prepared for the purpose of demonstrating the effects of heat upon the stability of the inhibitor, if present in the immature bean.

All plant extracts were freshly made just prior to time of preparing the digests for incubation.

Casein solution. An approximate 3 percent soluble casein solution was prepared by dissolving 60 grams of powdered commercial casein (Cenco) in a dilute sodium carbonate solution (6 grams of sodium carbonate in 1934 ml. water). The pH of the casein solution was approximately 9.0. The final pH was adjusted as described later under method.

Buffer. A neutral phosphate buffer was prepared by mixing 389.0 ml. of a 1 M solution of KH_2PO_4 with 611.0 ml. of a 1 M Na_2HPO_4 solution.

Trypsin solution. A crude trypsin solution was prepared by blending 0.5 gram trypsin powder (Cenco) in 99.5 ml. distilled water in a Waring blendor for five minutes. The trypsin and casein solutions were prepared and stored at 36° F. on the day preceding the test

periods. These solutions were warmed to 37° before introducing them into the digest systems.

The action of trypsin upon casein in the presence and absence of soybean plant extracts was studied by the use of replicate digests. The undigested protein remaining after a given length digestion period was used as the basis for measuring and comparing rates of tryptic action.

For each of the four stages of soybean plant development studied, there were incubation periods of 0, 1, 2, 3, 4, and 5 hours with the exception of stage 3 (see figure 1). For each incubation period con-

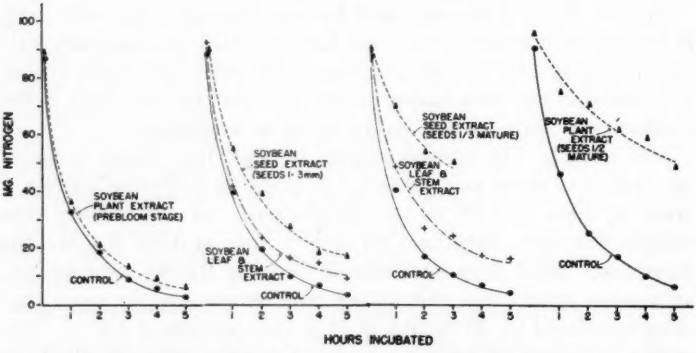

Figure 1. The influence of raw extracts of green soybean forage upon the proteolytic activity of trypsin is indicated by these curves.

ducted, five control digests and an equal number of test digests containing the plant, leaf-stem, or seed extracts were prepared.

The digest systems were made up in 125 ml. beakers. These systems were prepared by introducing into each beaker 25 ml. of casein solution and 5 ml. of phosphate buffer. To the test systems were added 3 ml. of soybean plant or seed extract and to the controls were added 3 ml. of distilled water. To each series of control and test systems, with the exception of the non-incubated series, was then added 2 ml. of trypsin solution. The final pH of the systems that were to be incubated was 8.5. (This pH was attained by readjusting the pH of the casein to an approximate pH of 10.0, depending upon the somewhat variable pH of the plant extracts used). In the case of the non-incubated systems, trypsin was added after the introduction of a sufficient 10 percent

acetic acid solution to bring the system to a pH of 4.8. The systems were incubated at 37° C.

After incubating for a given period, the systems were acidified in the same manner as was described for the non-incubated systems. Any protein escaping tryptic action was precipitated and filtered on Munktells' filter paper, no. OK. The coagulant was washed three times with cold distilled water and then analyzed for total nitrogen by the Kjeldahl Gunning Arnold method (1945).

A statistical study of the data was made by an analysis of variance according to Snedecor (1946). Before the analysis of variance was made, six missing values, namely; 1 in the preblooms, 3 in the seeds just formed, and 2 in the seeds one-third mature stages of development, were supplied by the method of Yates (1943). In no case was more than one observation missing from any one of the subclasses which normally consisted of five observations. At the seeds just formed, and seeds one-third formed stages there were two degrees of freedom for treatments. This resulted from having one treatment at each stage made up of an extract from seeds alone and another treatment made up of an extract from leaves and stems. Under treatments for these two stages an X (treated vs. control) and a Y (seed vs. stem extract) mean square was calculated. In showing the magnitude of the influence of these treatments on protein hydrolysis, the components of variance were calculated by equating the sample mean squares to their expected values.

Results and Discussion

The data for the comparative rates of protein digestion as limited by tryptic activity are graphically shown in figures 1 and 2. Each point on any one curve unless otherwise indicated represents the mean total nitrogen value for five observations made at the end of any one given incubation period. Table 1 summarizes the analysis of variance of the nitrogen values for each of the four stages of plant development as well as the effects of heat upon the stability of the seed extract.

As shown by the analysis of variance the main effect for treatments was not significant at the prebloom stage, even though the values in figure 1 suggest that a slight inhibition of tryptic action may have occurred. At the other three stages of development the treatment mean squares were significant at the 1 percent level of probability. Further study of the nitrogen values as shown in figures 1 and 2 further revealed

a highly significant interaction between hours and treatment for each of the four stages of plant development tested. The same may be said for the hour-main effects. The treated vs. controls (X) and seed vs. stem extract (Y) mean squares were significant at those stages where the plants were divided into seeds and leaf-stem portions. This showed that protein hydrolysis progressed more slowly in the soybean-treated systems than in the controls and that hydrolysis was retarded more by seed extracts than by the leaf-stem extracts.

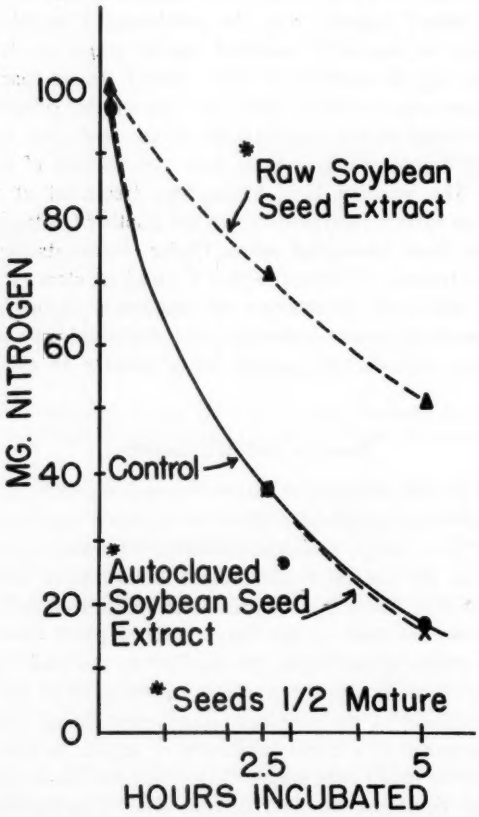

Figure 2. Here is shown the comparative effects of a raw and a heated extract of the immature soybean upon the proteolytic action of trypsin.

TABLE 1. ANALYSES OF VARIANCE FOR THE NITROGEN VALUES AT VARIOUS STAGES OF SOYBEAN PLANT DEVELOPMENT

	Prebloom stage		Seeds just formed		Seeds 1/3 formed		Seeds 1/2 formed		Effect of heat	
Source	Degrees freedom	Mean square	Degrees freedom	Mean square	Degrees freedom	Mean square	Degrees freedom	Mean square	Degrees freedom	Mean square
Total	58		85		87		59		43	
Treatments	1	85.0	2	1238.0**	2	4306.0**	1	19838.0**	2	2791.0
X	0		1[1]	1547.0**	1[1]	5128.0**	0		1[3]	5582.0*
Y	0		1[2]	930.0**	1[2]	3485.0**	0		1[4]	.03
Hours	5	9920.0**	5	13816.0**	5	11528.0**	5	5656.0*	2	20602.0**
Treatments x hours	5	14.0**	10	61.0**	10	229.0**	5	708.0**	4	544.0**
Error	47	.19	68	1.67	70	8.24	48	9.73		1.43

* Significant at 5% level of probability.
** Significant at 1% level of probability
[1] Treated vs. control
[2] Seed vs. stem extracts
[3] Raw vs. control
[4] Heated vs. control

The relative importance of the various sources of variation at the different stages of soybean plant development is shown in table 2. A very large percentage of variance was due to hours in the earlier stages, but this percentage decreased markedly in the later stages. Equally important, as shown in table 2, was the increased variance for treatments from almost 0 percent in the prebloom stage to 50 percent for the stage in which seeds were one-half formed.

The effects of heat upon the stability of the inhibitor in the immature seeds was demonstrated by heating the immature seed to 120° C. for 30 minutes at 15 pounds pressure. With this treatment the antiproteolytic properties were destroyed as is shown in figure 2 and table 1.

TABLE 2. THE COMPONENTS AND PERCENTS OF TOTAL VARIANCE FOR EACH STAGE OF FORAGE DEVELOPMENT

	Prebloom stage		Seeds just formed		Seeds 1/3 formed		Seeds 1/2 formed	
Source	Variance	%	Variance	%	Variance	%	Variance	%
Treatments	2.37	00.2	39.25	4.0	135.93	14.4	637.66	49.7
Hours	990.61	99.5	917.00	94.6	753.28	80.0	494.77	38.6
Treatment x hours	2.71	00.3	11.79	1.2	44.05	4.7	139.69	10.9
Error	.19	Trace	1.67	0.2	8.24	0.9	9.73	0.8

From these data it appears that extracts of fractions from soybean forage, taken at different stages of development when in the presence of trypsin affected casein hydrolysis. Also it appears that the trypsin inhibiting factor increased in concentration as the soybean plant developed. Furthermore the concentration of the inhibiting factor was much greater in the seeds than in the leaf-stem fraction. To be able to explain the exact nature of the inhibition of trypsin by green soybean forage extracts as shown by these studies would require further investigation.

It would seem that in the light of the evidence herein presented further study of the soybean as a forage for livestock feeding would be justified.

Summary and Conclusions

The effects of extracts of green soybean forage fractions on the proteolytic action of trypsin were studied. Under the conditions of

these experiments there was no significant inhibition of the activity of trypsin when in the presence of an extract from soybean forage in the prebloom stage of development. However, the existence of a trypsin inhibiting factor in soybean forage was observed at the onset of seed formation. The proteolytic action of trypsin upon casein was retarded in some manner by extracts from the vegetative portion of green soybean plants from which seeds had been removed. The extent to which trypsin action was inhibited was much greater in the presence of seed extracts than that of leaf-stem extracts. The concentration of the inhibiting factor showed a tendency to increase as the soybean plant became more mature. The trypsin inhibiting factor in the immature seed was inactivated by heating to a temperature of 120° C. at 15 pounds pressure.

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THE EFFECT OF DRYING TEMPERATURE UPON THE NUTRITIVE VALUE AND COMMERCIAL GRADE OF CORN[1]

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CORN growers are sometimes confronted with difficulty in the safe storage of their grain crop because of high moisture content at time of harvest. This condition is especially pronounced in years of late maturity, early frost, early harvest or weather conditions that are unfavorable for natural field drying. A study was therefore undertaken of the feasibility of drying such ear corn at maturity by heated air under forced draft. The chief objectives were to determine the comparative effects of seven distinct temperatures on market grade and nutritive value of the crop.

Cox, MacMasters and Hilbert (1944, 1945) pointed out that it is common knowledge among corn wet millers that great difficulty is experienced in processing corn that has been artificially dried at 180° to 200° F. These workers found that prime quality starch could not be obtained from a sample of corn dried under these conditions as it was impossible to separate all of the gluten from the starch.

Adams, Stark and Kolachov (1943) studied the effect of kiln drying upon the fermentable carbohydrate content of corn and reported, "Kiln drying of corn results in a 2 to 3 percent decrease in alcohol yield. The action of heat on the fermentable carbohydrate portion of the grain has not been definitely characterized but is probably a formation of a certain amount of unfermentable dextrine."

Simms (1949) reported that mature shelled corn of moderately low moisture content may be dried in hot air at temperatures up to 190° F. for 10 hours without significant loss of feeding value.

Mitchell, Hamilton and Beadles (1949) tested the effects of the processes involved in the manufacture of corn flakes upon the nutritive value of corn protein. In the preparation of the flakes, the corn was cut, water was added and the corn cooked for 20 to 45 minutes at a steam pressure of 45 to 50 pounds per square inch. The final toasting

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process raised the flakes to a temperature of 350° to 400° F. for a period of three to four minutes. A comparison of the unprocessed corn with the flakes, prior to the toasting process, showed a drop in the digestibility of the protein from 90.0 percent to 75.8 percent. There was also an indication of a small drop in the biological value. The overall heat damage averaged 20.1 percent. During the toasting process the protein digestibility increased on an average from 73.8 to 79.8 percent.

Studies with rats and swine reported by the U.S.D.A. (1950) indicated that the feeding value of soft corn is reduced when drying temperatures go above 135° or fall below 95° F. The damage below 95° was regarded as possibly due to enzyme spoilage during very slow drying periods. Temperatures below 95° affected the lysine and tryptophan content of the corn. Drying by hot air produced essentially the same results as drying by a high frequency electric current.

Experimental Procedure

Source and Drying of Grain

Within a period of 19 days after maturity (34 percent kernel moisture) during each of three years, 1946–1948, six 2.5-bushel lots of U. S. 13 hybrid yellow dent ear corn were husked from an Experiment Station field and dried at six temperatures (table 1) ranging from 120° to 240° F., respectively. In addition a seventh "check" lot of ear corn was dried at a mean room temperature of 80° F. The purpose of the drying was to lower the moisture content in all lots of the grain to the safe storage content of about 14 percent. This was accomplished rather uniformly (table 1) by means of an especially constructed electric drier, containing five 24 x 36 inch mesh bottom trays, each having a capacity for approximately one bushel of ears in a single layer. The trays were spaced one above the other so as to allow free circulation of air heated to the desired temperature by a thermostatically controlled 7200-watt heating unit. The air velocity averaged 170 feet per minute over the corn, and its temperature throughout the drier was measured with iron-constantan thermocouples and a Brown potentiometer. Mercury thermometers were used in several locations as additional checks.

Since only one drier was available, periods of eight to ten days were required in the different years to gather and dry all six lots of corn. As a consequence, these differed somewhat in initial moisture content but

all exceeded 27 percent. The "check" lot of ear corn was composited from half-bushel quantities of corn that were gathered as part of the other six harvests and placed immediately on a wire rack in the seed room for natural drying.

TABLE 1. EFFECT OF DRYING TEMPERATURE UNDER FORCED DRAFT ON RATE OF DRYING AND MARKET GRADE OF U. S. 13 HYBRID CORN

3 years, 1946–1948[1]

Drying temp.	Duration drying period	Moisture content		Commercial grade[2]
		Initial	Final	
(F.)	(Hours)	(pct.)	(pct.)	(No.)
		Year 1946		
80	312	31.8	17.9	1
120	49	28.1	14.0	1
140	26	30.3	14.8	1
160	13	36.5	17.5	1
180	13	35.6	15.8	1
200	9	32.2	14.3	4[3]
240	5.5	27.4	14.7	5[3]
		Year 1947		
80	312	32.9	14.6	1
120	33.5	33.7	15.1	1
140	26	35.3	14.5	1
160	12	28.2	14.6	1
180	22	33.1	14.1	2
200	10.5	36.2	14.6	1
240	4	30.8	14.5	Sample[3]
		Year 1948		
80	312	29.9	14.5	1
120	38	31.9	15.0	1
140	24	27.9	14.6	1
160	14	29.6	14.4	1
180	12	34.0	15.0	1
200	7	29.8	14.9	1
240	6.8	31.2	13.2	4[3]

[1] The first harvest dates were September 28, 11 and 15 in 1946, 1947 and 1948, respectively.
[2] The corn was graded according to official standards by David A. Sander of the Department of Agronomy. Moisture was not considered as a grading factor.
[3] Heat damaged.

As soon as the check lot had reached the desired moisture content, all of the corn was shelled and stored in cloth bags at 82° F. until fed. The moisture determinations were made with a Steinlite tester and the initial moistures were oven-checked.

Feeding Tests

The comparative nutritive values of the seven lots of corn were determined annually by feeding them to rats in especially designed rations (table 2) according to standard procedure. The management of the rat colony was the same as that outlined in a previous study (1945) except as otherwise indicated. Pied male and female rats, 23 to 27 days

TABLE 2. INGREDIENTS OF THE TWO TYPES OF RATIONS IN WHICH CORN WAS USED AS A SOURCE OF ENERGY AND AS A SOURCE OF PROTEIN

Corn as source of energy		Corn as source of protein	
Ingredients	Percent of ration	Ingredients	Percent of ration
Corn (one of 7 lots)	76	Corn (one of 7 lots)	90
Linseed oil meal	16	Salt mixture[1]	4
Tankage	5	Cod liver oil concentrate	2
Alfalfa (ground)	2	Vegetable Oil[2]	4
Sodium Chloride	0.5	Riboflavin[3]	—
Calcium Carbonate	0.5	Vitab[3]	—
Cod-liver oil concentrate	2.0		

[1] Salt mixture No. 2 (U. S. Pharmacopia, 1934) was used.
[2] The Vegetable oil was "Primex" manufactured by Procter and Gamble, Cincinnati, Ohio.
[3] A mixture of 294 mcg riboflavin and 1.2 ml Vitab, diluted with distilled water, was fed separately in Syracuse watch glasses.

old and weighing between 40 and 49 grams, were placed in individual cages which were fitted with false bottoms of 13 mm. screen. All rats received distilled water to which iodine had been added. The animals were weighed weekly and individual food consumption records were kept. To reduce variability in results due to inherent differences, 18 replicate rats were fed each ration in each of the three years, 1946–1948.

The Rations

Two types of rations were prepared for the purpose of comparing the seven lots of corn both as a source of energy and as a source of protein. This required two distinct basal rations, in conjunction with each of the seven lots of corn dried at the various temperatures. Thus, there were 14 rations as described in table 2, each fed annually to 18 rats. The seven rations in which the corn served as a source of energy were

included in one test each year, followed by a corresponding test in which corn was the only source of protein.

The corn was prepared, for mixing into the basal ration, by grinding in a hammer mill. There was a loss of moisture as a result of this grinding. Since the various lots of corn did not all contain the same moisture percent (table 1), a moisture determination was made by the A.O.A.C. method 1945, just before the rations were mixed. The ground corn was then added on the basis of its dry matter content and the ration blended in a mechanical mixer. The rations were freshly prepared at approximately weekly intervals and stored at 38° to 40° F. until fed.

Whenever corn was ground or whenever a ration was mixed, a sample of the ground grain or mixed ration was set aside for chemical analysis. At the end of the experiment, the samples from a given lot of corn or ration were pooled and composite samples analyzed by A.O.A.C. methods (1945) for moisture, fat, fiber, ash, protein and nitrogen free extract.

Results

Market Grade of Corn as Influenced by Drying Temperature

The average initial and final moisture percentages and the resulting commercial grades of corn are shown in table 1 for the seven different drying temperatures and for each of the three years. There were no visible adverse market effects that could be ascribed to the drying procedure except for heat damage at 240° F. in all years and 200° F. in one year.

Feed Constituents of Grain in Relation to Drying Temperature

No important differences in the feed constituents of the grain resulted from drying at any of the various temperatures, insofar as could be measured by standard chemical procedure. As shown in table 3, the grain used in the feeding rations as a source of protein was one to two percent lower in moisture percentage because of somewhat longer storage, and this was offset by slightly higher percentages of the other constituents. This difference was recognized in preparing the rations.

Since there were no significant changes in the percentages of the various feed constituents in the corn as a result of difference in drying temperature, it follows that there also would be none in the component rations having the same other basic composition (table 4). The differ-

ences in feed constituents appear too small to account for the differences in growth of the rats.

Gain in Weight of Rats in Relation to Drying Temperature of Corn

The seven groups of rats fed either type of ration had essentially the same initial weight and all gained at a rather steady rate. However,

TABLE 3. EFFECT OF DRYING TEMPERATURE ON FEED CONSTITUENTS OF U. S. 13 HYBRID CORN. AVERAGE FOR 3 YEARS, 1946–1948

Drying temp.	Feed constituents					
	Moisture[1]	Ash	Fat	Protein	Nitrogen free extract	Crude fiber
(° F.)	(pct.)	(pct.)	(pct.)	(pct.)	(pct.)	(pct.)
	Corn used as source of energy					
80	8.5	1.5	4.4	10.2	73.4	2.0
120	8.5	1.5	4.5	10.4	73.1	2.0
140	8.1	1.5	4.5	10.3	73.6	2.0
160	8.9	1.5	4.4	10.5	72.7	2.0
180	7.9	1.4	4.6	10.2	73.8	2.1
200	7.5	1.5	4.4	10.5	74.1	2.0
240	7.5	1.5	4.6	10.5	74.3	2.1
	Corn used as source of protein					
80	6.6	1.5	4.6	10.6	74.6	2.1
120	6.5	1.5	4.5	10.7	74.7	2.1
140	6.5	1.5	4.7	10.6	74.6	2.1
160	6.4	1.5	4.6	10.8	74.6	2.1
180	6.3	1.5	4.8	10.5	74.8	2.1
200	5.9	1.5	4.7	10.8	75.0	2.1
240	5.7	1.5	4.8	10.7	75.3	2.0

[1] The moisture content of grain used as a source of protein is somewhat lower because of several weeks longer storage.

those fed the corn as the source of protein averaged a slightly heavier initial weight than those receiving it as the source of energy. This was intentional as it was thought that protein-deficient rats would benefit by somewhat greater maturity.

Gains in weight were distinctly higher for rations in which the corn served as a source of energy than for rations in which the corn served as a source of protein. This was expected since the latter rations contained less protein. There was a positive tendency for reduced gains from corn dried at temperatures above 140° F. when this corn was used as a source of energy. This relation is readily seen in table 5, in which

the actual and relative gains are shown after feeding the specific rations for four and eight weeks. The inferiority of gain resulting from the feeding of grain dried at the temperatures above 140° F. is statistically significant in all cases.

In those rations in which corn dried at a temperature of 140° F. or less was used as a source of protein, the deviations from the check in

TABLE 4. FEED CONSTITUENTS OF THE 14 RATIONS PREPARED FOR THE FEEDING TESTS OF U. S. 13 HYBRID CORN THAT HAD BEEN DRIED AT SEVEN DIFFERENT TEMPERATURES. 3 YEAR AVERAGE, 1946–1948

Drying temp.	Feed constituents					
	Moisture	Ash	Fat	Protein	Nitrogen free extract	Crude fiber
(° F.)	(pct.)	(pct.)	(pct.)	(pct.)	(pct.)	(pct.)
	Corn used as source of energy					
80	6.9	4.1	6.6	16.0	63.0	3.4
120	7.8	4.2	6.5	15.9	62.2	3.4
140	7.1	4.1	6.6	15.7	63.2	3.3
160	8.1	4.1	6.7	15.7	62.1	3.3
180	7.7	4.0	6.6	15.8	62.5	3.4
200	6.9	4.0	6.7	16.2	62.8	3.4
240	6.5	4.2	6.6	16.0	63.4	3.3
	Corn used as source of protein					
80	6.8	3.4	9.7	9.6	68.5	2.0
120	6.8	3.4	9.9	9.6	68.5	1.8
140	6.4	3.4	9.8	9.6	68.9	1.9
160	6.7	3.4	9.8	9.7	68.5	1.9
180	6.4	3.4	9.9	9.4	69.1	1.8
200	6.2	3.4	9.7	9.7	69.1	1.9
240	5.8	3.4	9.9	9.6	69.4	1.9

amount of gain in weight were not statistically significant. A 7 or 8 percent lower gain in weight from corn dried at 140° F. compared with the check in the corn for energy tests was significant at the 5 percent level.

As averages for rations containing corn dried at temperatures above 140° F., the gains at four and eight weeks were respectively 21 and 23 percent lower than for the check in tests using the corn as a source of protein. In the corn as a source of energy tests, corresponding reductions were 11 and 9 percent.

Feed consumed by Rats in Relation to the Drying Temperature of Corn

A summary of the feed consumption and of the gains per unit of feed consumed is presented in table 6 for the various rations. In tests using the corn as a source of energy, there was a distinct though non-significant trend for reduced total feed consumption during eight weeks of feeding rations containing corn dried at 140° F. or higher. This

TABLE 5. EFFECT OF THE DRYING TEMPERATURE OF U. S. 13 HYBRID CORN UPON THE GAIN IN WEIGHT OF RATS DURING FOUR AND EIGHT WEEK PERIODS OF FEEDING. AVERAGE FOR 3 YEARS, 1946–1948[1]

Drying Temp. of corn in rations	Gain in weight per rat		Relative gains in weight (% of check)	
	4-weeks	8-weeks	4-weeks	8-weeks
(° F.)	(Grams)	(Grams)		
	Corn used as source of energy			
80 (check)	60.4	118.7	100	100
120	57.9	114.9	95.9	96.8
140	55.4	110.5	91.7*[2]	93.1*
160	55.2	108.9	91.4*	91.8*
180	53.0	109.0	87.7**	91.8*
200	53.6	107.5	88.7**	90.6**
240	53.6	108.4	88.7**	91.3*
	Corn used as source of protein			
80 (check)	18.4	37.6	100	100
120	19.9	38.8	108.2	103.2
140	19.1	38.1	103.8	101.3
160	15.2	31.0	82.6**	82.4**
180	13.3	25.6	72.3**	68.1**
200	14.9	29.9	81.0**	79.5**
240	14.7	29.9	79.9**	79.5**

[1] Averages for 18 replicate rats annually.
[2] Standard errors were computed according to the method of Snedecor, 1940.
* Deviation from check (80° F.) significant at 5 percent level.
** Deviation from check (80° F.) significant at 1 percent level.

reduction averaged 5 percent and compares with an 8 percent lower gain in weight of rats as shown in table 5. This is regarded as evidence of a reduction in the palatability of corn by heating at temperatures of 140° F. or higher. Gains in weight per unit feed consumed were very uniform for all rations.

In tests using the corn as a source of protein, the amount of feed consumed during eight weeks averaged 5 percent less than the check

for rations containing corn dried at 160° F. or higher temperature. This compares with a 23 percent lower gain in weight of rats (table 5). Gains in weight per unit feed consumed were reduced 18 percent as an

TABLE 6. EFFECT OF THE DRYING TEMPERATURES OF U. S. 13 HYBRID CORN UPON THE AMOUNT OF FEED CONSUMED BY RATS DURING FOUR AND EIGHT WEEK PERIODS OF FEEDING. AVERAGE FOR 3 YEARS, 1946–1948[1]

Drying temp. of corn in ration	Feed consumption per rat		Relative feed consumption[2]		Gain in weight per gram feed consumed	
	4-weeks	8-weeks	4-weeks	8-weeks	4-weeks	8-weeks
(° F.)	(Grams)	(Grams)	(Grams)	(Grams)	(Grams)	(Grams)
	Corn used as source of energy					
80 (check)	224.0	517.8	100.0	100.0	0.27	0.23
120	220.1	510.8	98.3	98.6	0.26	0.22
140	210.2	495.0	93.8	95.6	0.26	0.22
160	208.7	488.1	93.2	94.3	0.26	0.22
180	213.8	499.0	95.4	96.3	0.25	0.22
200	205.0	486.5	91.5	94.0	0.26	0.22
240	208.1	491.6	92.9	94.9	0.26	0.22
	Corn used as source of protein					
80 (check)	158.2	344.0	100.0	100.0	0.12	0.11
120	163.8	360.6	103.5	104.8	0.12	0.11
140	164.7	359.0	104.1	104.4	0.12	0.11
160	151.9	324.6	96.0	94.4	0.10	0.10
180	148.6	314.3	93.9	91.4	0.09	0.08
200	151.8	331.3	96.0	96.3	0.10	0.09
240	155.3	339.6	98.2	98.7	0.09	0.09

[1] Averages for 18 replicate rats annually.
[2] None of the deviations from check (80° F.) in feed consumption proved statistically significant except at the 5 percent level for corn dried at 180° F. and fed as a source of protein for eight weeks.

average for rations containing corn dried at temperatures of 160° F. or more. This is evidence that the feeding value of the protein of corn has been materially reduced by drying temperatures above 140° F.

Supplementing corn with casein as sources of protein

After eight weeks of feeding the seven rations in which corn was the only source of protein (table 6), purified casein was added to bring each ration up to 18 percent protein content. The same rats were then continued an additional two weeks on the modified ration (table 7). During this two week period with a balanced ration, the weekly gain was

increased six fold. These results indicate that the deficiency of protein was primarily responsible for the small gains obtained from rations in which the corn served as the only source of protein although the decrease in the value of corn as a source of energy may have been partially responsible.

Summary

Mature ear corn was reduced in kernel moisture content from 27 percent or more to a safe storage value of about 14 percent by heated air under forced draft at temperatures ranging from 240° to 120° F. in four to 49 hours.

The market grade of corn was not affected by drying temperatures up to 180° F. Drying at 200° F. and 240° F. in one year 1946 lowered the grade to four and five respectively, because of heat damage. In two other years, 1947 and 1948, a 240° F. temperature lowered the grade to sample and grade 4 as a result of heat damage.

Drying at seven different temperatures ranging from 80° to 240° F. did not materially affect the proportion of the various feed constituents of corn on a comparable moisture basis. The nutritive value of the protein, however, was affected adversely at 160° F. or more as evidenced by reductions of 18 to 32 percent in the weight gain of rats fed individually

TABLE 7. GAINS IN WEIGHT OF RATS FED FOR EIGHT WEEKS WITH RATIONS IN WHICH U. S. HYBRID CORN DRIED AT VARIOUS TEMPERATURES WAS THE ONLY SOURCE OF PROTEIN, FOLLOWED BY TWO WEEKS IN WHICH CASEIN WAS ADDED TO RAISE THE PROTEIN CONTENT TO 18 PERCENT. AVERAGE FOR 3 YEARS, 1946–1948

Drying temp.	Gains with corn as only source of protein		Gains following addition of casein to same ration in 2 succeeding weeks	
	8-week period	Average per week	9th & 10th Weeks	Average per week
(° F.)	(Grams)	(Grams)	(Grams)	(Grams)
80 (check)	37.6	4.7	49.7	24.9
120	38.8	4.9	46.8	23.4
140	38.1	4.7	52.8	26.4
160	31.0	3.9	51.9	26.0
180	25.6	3.2	47.8	23.9
200	29.9	3.7	51.7	25.9
240	29.9	3.7	45.9	23.0
Ave.	33.0	4.1	49.5	24.8

for eight weeks with especially designed rations in which the corn was the only source of protein. These higher temperatures also reduced the amount of feed consumed an average of 5 percent under that of the check dried naturally at 80° F. There was a corresponding reduction of 18 percent in the amount of gain per unit feed consumed.

When corn dried at various temperatures was tested as a source of energy in special rations fed to rats, its energy value was significantly reduced by 140° F. or higher temperatures. This amounted to 8 percent less gain after eight weeks of feeding. A corresponding non-significant reduction in the amount of feed consumed averaged 5 percent. Grain dried at the higher temperatures was apparently somewhat less palatable, although the gain in weight per unit feed consumed was but slightly affected as compared with the naturally dried check.

As a general practice, drying corn at temperatures above 140° F. would be objectionable from the standpoint of reduced nutritive value. This reduced feeding value may not be apparent in the feeding of hogs when a protein-mineral-vitamin supplement is added to the ration or in the feeding of the mature ruminant which has the ability to utilize less desirable feeds due to the microorganisms of the digestive tract. However, since over-heating corn lowers its market grade and impairs its milling quality in addition to reducing its intrinsic nutritive value, excessive drying temperatures should be avoided.

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MEMORIALS

W. T. COBB

W. T. Cobb was born on a farm in DeKalb County, Georgia. At an early age the family moved to Slaughter, Louisiana, where his father owned and operated a cotton and cattle farm. He received his B.S. degree from Louisiana State University in 1928 and his M.S. degree in 1936.

Tony, as he was affectionately called, entered agricultural extension work shortly before World War I, and was one of the pioneers in that field, serving successfully as county agent. Despite the difficulties of cattle production, Cobb was among those who insisted that someday the cattle tick would be eradicated, that good breeds could be introduced and that Louisiana could become a great cattle state. In 1920 he became assistant to the Superintendent of the U. S. Department of Agriculture's Iberia Livestock Experiment Station, where he continued to learn more and more about producing cattle under Louisiana conditions. Later, he served as Superintendent from 1924 to 1930.

In 1930 he returned to the Agriculture Extension Service as a beef cattle specialist, which he held until his death. He was one of the founders of the present Louisiana Cattlemen's Association. Together with W. C. Abbott, 4-H club leader, and others, he started the first junior livestock show for boys and girls in 1936.

Cobb was also well known as a judge of all types of beef cattle and served at all of the shows in Louisiana at various times, as well as at many major shows in other states. Dean of the College of Agriculture, J. G. Lee, Jr., says that he considered Cobb one of the best informed men in America on all phases of livestock.

It is not surprising that, in recognition of these facts, the Louisiana Cattlemen's Association should undertake to establish a living memorial to W. T. Cobb—not a monument or a building dedicated to him, but a memorial fund to be used to help carry on the work to which he devoted his life.

JOEL SIMMONS COFFEY

Born on a farm near Rugby, Indiana, on December 8, 1885, Joel Coffey was from a family of six children. He farmed for two years until 1908 when he enrolled at the University of Illinois College of Agriculture. In 1912 he received his B.S. degree in agriculture and then became a teacher of animal husbandry at Purdue University for two years. He later joined the teaching staff of Ohio State University and taught summer school at the University of Iowa.

Mr. Coffey completed his 37th year of service at Ohio State University September 1, 1951. He had taught freshman animal husbandry and was in charge of swine production operations.

He has served as a judge of swine at State Fairs and National exhibitions in Ohio, Indiana, Illinois, Wisconsin, Kentucky, Pennsylvania, Michigan, New Jersey, California, and Maryland. He also judged at the International Livestock Show at Chicago, the Pacific International at Portland, the National Barrow Show at Austin, Minnesota and at three national swine shows.

Mr. Coffey was a member of University Lodge of Masons No. 631, Acacia and Alpha Zeta fraternities, and the American Society of Animal Production. He served as secretary of the Ohio Swine Producers Association for many years,

was a regular contributor to several swine publications, and was co-author of a book entitled "Livestock Management." He was a member of the North Broadway Methodist Church.

Mr. Coffey leaves his wife, Dorothy Neff Coffey, a graduate of Ohio State University in 1923; a son, Robert J. Coffey, a graduate of Ohio State in 1949; and a daughter, Margaret Joan, a graduate of Ohio State in 1944. He also had four grandchildren.

NEWS AND NOTES

The forty-fourth annual meeting of the American Society of Animal Production will be held in the Hotel Sherman in Chicago, Illinois, on November 28 and 29, 1952, with a dinner for the honored guest on November 30. Members planning to present papers are reminded that titles must be in the hands of the secretary, Dr. J. I. Miller, Department of Animal Husbandry, Cornell University, Ithaca, N. Y., by July 20, following which abstracts must be filed with him by August 15.

Nominations for the Morrison Award, in writing, are due in the Secretary's Office by June 15. All members of the Society except members of the Executive Committee and the Award Committee are eligible to make one nomination. The rules are the same as last year and are essentially the same as published in the JOURNAL OF ANIMAL SCIENCE, Vol. 5, pp. 236–237, 1946. All nominations must be accompanied by a brief biography of the nominee and a list, together with copies, of publications concerning the research, and a statement of the importance to the livestock industry of the work upon which the nomination is based.

Nominations for American Feed Manufacturers Award should be sent to the Secretary's Office by June 15. Research published in 1950 and 1951 will be considered for the 1952 award. The rules governing this award are unchanged from last year and are stated in the JOURNAL OF ANIMAL SCIENCE, Vol. 7, pp. 257–258, 1948.

A. D. Weber, Associate Director of the Agricultural Experiment Station, Kansas State College has been chosen as the honor guest for the American Society of Animal Production at its meeting November 28 and 29. His portrait will be presented to the Saddle and Sirloin Club at the time of the banquet in his honor on November 30. R. F. Cox is chairman of the committee in charge of arrangements for the event.

The annual meetings of the Western Section, American Society of Animal Production, will be held at the University of California, Davis, June 23 and 24. Officers for the Western Section are: L. E. Harris (Utah), president; J. L. Van Horn (Montana), vice-president; and H. Heitman (California), secretary-treasurer. The annual meeting of the American Dairy Science Association is also being held in Davis, June 24 to 26, giving those in attendance an opportunity to attend both meetings. Information may be obtained from the secretary, H. Heitman, University of California, Davis, California.

The committee on Ralston-Purina Fellowship Awards has selected the winners for the school year 1952–53. The principals named for Animal Husbandry were Raymond F. Sewell of Florida, James M. Fransen of Oklahoma, and Daniel W. Beardsley of Florida. The institutions at which these men preferred to work were Cornell, Purdue, and Illinois, respectively. The Dairy Husbandry principals were Harold A. Ramsey of Kansas, and Walter E. C. Moore of New Hampshire. These men preferred North Carolina State and Wisconsin, respectively. The principals for Poultry Husbandry were Edward C. Naber of Wisconsin and Ernest Williams of Louisiana. The University of Wisconsin and Louisiana State were the institutions at which these men preferred to continue their work. Alternates were Lawrence R. Berg, of Washington, Joseph E. Bertrand of Louisiana, Harvey L. Bumgardner of North Carolina, Kendall L. Dolge of Connecticut, Joseph F. Fontenot of Louisiana and LeRoy F. Nelson of Nebraska.

The nature of these awards and some of the rules governing them were published in the JOURNAL OF ANIMAL SCIENCE, 1951, Volume 10, page 307.

S. A. Asdell of Cornell University is to be the official representative of the American Society of Animal Production at the Second International Congress on the Physiology and Pathology of Animal Reproduction and Artificial Insemination to be held in Copenhagen July 7–11.

A new and greatly expanded edition of Telesforo Bonadonna's book, *Zootecnica Speciale,* has been published by the Instituto Editoriale Cisalpino, Milan, Italy. The first volume deals with the distribution of livestock in general, artificial insemination and the registration of domestic animals. A wealth of information on breed societies and their policies is given, together with sample registration forms for many. The second volume deals with cattle and horses, giving statistics on distribution, yields and rates of growth for the principal breeds of the world. The third includes swine, sheep, poultry and fur animals. Management and feeding are dealt with somewhat briefly.

Raising swine by Deyoe and Krider was published by McGraw-Hill Book Co., New York, in January, 1952. The book is intended to provide information helpful to practical swine raisers. Included is material on recent developments in swine nutrition, selection and improvement, and on approved practices. Special emphasis is placed on new types of records to measure production and methods of using them to improve the swine enterprise.

Range management by Arthur W. Sampson was published by John Wiley and Sons, Inc., New York, in January, 1952. This comprehensive book aims to balance considerations on the care and management of range lands and its grazing animals with the sciences upon which sound practices must be based. It is organized in four parts: range management in perspective, native range forage plants, improvement and management of range and stock, and protection of range resources and range livestock.

Dairy Cattle Breeding by L. O. Gilmore was published by J. B. Lippincott Company, Chicago in February, 1952. The aim of the author has been to record the present status of the knowledge of breeding dairy cattle primarily for students in agricultural colleges. The inheritance of various simple characters and of certain aspects of milk secretion and other physiological functions are included. Attention is also given to systems of mating, reproduction and artificial insemination.

Understanding Heredity by Richard B. Goldschmidt was published by John Wiley & Sons, Inc., New York in February, 1952. This book is an introduction to genetics. A simple presentation of the facts of heredity is intended following which the author deals briefly with advanced genetic problems of today.

Kansas State College, Argonne National Laboratory and the Isotopes Division of the United States Atomic Energy Commission will sponsor a two-and-a-half-day conference on The Use of Isotopes in Plant and Animal Research, June 12–14, 1952 at Kansas State College, Manhattan, Kansas. A detailed program will be available in March. Persons wishing to receive it, together with information on housing, should write Director R. I. Throckmorton, Kansas Agricultural Experiment Station, Manhattan, Kansas.

Edward James Wilford, Professor of Animal Husbandry, University of Kentucky, died on January 4, 1952.

James O. Grandstaff has transferred from the Bureau of Indian Affairs, Department of the Interior, Fort Wingate, New Mexico, to the Office of Experiment Stations, Department of Agriculture, Washington, D. C., as Experiment Station Administrator in the field of animal husbandry. He has been director of the Southwestern Range and Sheep Breeding Laboratory since 1944.

Lyle McCartney, former Asst. County Agent, Elko County, Nevada, was appointed Associate in Range Management at the Nevada Experiment Station, Dec. 1, 1952.

James F. Kidwell, Animal Husbandman at the Louisiana Experiment Station has gone to Nevada to head up the Department of Animal Husbandry. Richard A. Damon, research fellow at University of Minnesota has taken Kidwell's place at Louisiana.

R. C. Carter, Animal Husbandman at the Virginia Experiment Station, left Virginia, Feb. 15, 1952 on educational leave for study at Iowa State College.

Francis N. Neville, Jr., was appointed Associate in Veterinary Science at the Nevada Experiment Station, March 1, 1952. Formerly on the staff of Texas A. & M., he enters this position after approximately two years in private practice.

E. W. Klosterman has gone from the North Dakota Experiment Station to the Ohio Experiment Station at Wooster to fill the vacancy resulting from the death of Paul Gerlaugh.

L. E. Washburn has been appointed head of the Animal Husbandry Department at the Colorado Experiment Station, relieving S. S. Wheeler, who is now Director of the Colorado Agricultural Experiment Station.

E. O. Anderson, Professor of Dairy Mfg. at the Agricultural Experiment Station, Storrs, Conn., has been given a year's leave of absence to serve under and MSA appointment as a Dairy Processing Specialist for Western Europe with headquarters in Paris.

Ralph E. Hunt, who has headed the Animal Husbandry Dept. at Virginia Polytechnic Institute since 1923, has given up administrative duties and is being succeeded by a former student, George W. Litton, who was Assoc. Animal Husbandman in the Dept.

J. A. Arey, Extension Professor in charge Dairy Extension at the North Carolina Agricultural Experiment Station was given recognition for outstanding service to the Dairy Industry of the South.

Randall R. Reed has been appointed research associate in Animal Husbandry at the New Jersey Experiment Station.

George E. Strum succeeds M. H. McDonald (who resigned recently to become field man for the American Hereford Cattle Association at Kansas City, Mo.), and will be in charge of statewide livestock improvement educational program of the North Dakota Agricultural College.

R. T. Clark, in charge beef cattle breeding research for the Animal Husbandry Division, Bureau of Animal Industry, U. S. Dept. of Agriculture, has been in Hawaii for the period of January 18 to May 30 at the invitation of the University of Hawaii, working with that institution and individual beef cattle breeders on the development of a regional beef cattle breeding project for the Territory.

Carl F. Sierk, Western Regional Beef Cattle Coordinator for the Animal Husbandry Division, Bureau of Animal Industry, USDA, with headquarters at Denver, Colorado, resigned effective Nov. 24, 1951, to become head of the Animal Husbandry Department of the University of Idaho, Moscow, Idaho. On Jan. 21, 1952, Carl B. Roubicek of the University of Wyoming became Western Regional Coordinator on the Denver staff.

A. L. (Fred) Baker, director of the U. S. Beef Cattle Research Station at Front Royal, Va., transferred to the Denver beef cattle office of the Animal Husbandry Division, Bureau of Animal Industry, USDA, as Animal Husbandman effective March 2, 1952. He will have as a principal responsibility the co-ordination of the Division's beef cattle-management research program. Bob M. Priode of the Vir-

ginia Agricultural Experiment Station, a member of the Front Royal staff has taken over as superintendent of the Front Royal station, which is operated in cooperation with the Virginia Agricultural Experiment Station.

Earl W. McComas, veteran Animal Husbandman in the beef cattle section of the Animal Husbandry Division, Bureau of Animal Industry, U. S. Dept. of Agriculture, transferred in March to the Agricultural Research Administration, Washington, D. C., to assist with the Point IV program.

Following the recent transfer of James O. Grandstaff, Director of the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico, to the Office of Experiment Stations of the U. S. Dept. of Agriculture at Washington, D. C., John A. Stoehr, Animal Husbandman of the U. S. Range Sheep Experiment Station, Dubois, Idaho, has been serving on temporary detail as acting director of the Fort Wingate Laboratory through the scoring, shearing, and lambing season of that station.

S. H. Work is now representing the Agriculture Division of the Food and Agriculture Organization in the North American Regional Office, 1344 Connecticut Ave. N.W., Washington, D. C. He was formerly with the Office of Experiment Stations in the subject-matter field of animal husbandry.

W. N. McMillen, formerly with Allied Mills is now Director of Feed Nutrition, A. E. Staley Manufacturing Co., Decatur, Ill.

F. I. Elliot, formerly Head of the Animal Industries, Department of the University of Connecticut is now manager of the Southeastern Artificial Breeding Association, Asheville, North Carolina.

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, double-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should contain a clear statement of the purpose of the investigation and its relation to other work in the same field, but long reviews should be avoided. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results, or their relation to some problem of the livestock industry. Each paper should end with a brief summary outlining the points proved in the investigation and their application, or their relation to a livestock problem.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. The author or his institution except in the case of invited review papers, will be required to purchase at least 100 reprints of his paper. There will be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsibility for the charges and for the reprints that will be ordered at that time.

6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts". *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest concerning the members, and other items of interest to the membership. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. L. E. Casida, Department of Genetics, University of Wisconsin, Madison, Wisconsin, or to the publishers: Boyd Printing Company, 372–374 Broadway, Albany 7, New York.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. J. I. Miller, Department of Animal Husbandry, Cornell University, Ithaca, N. Y., or to the publishers, Boyd Printing Company, 372–374 Broadway, Albany 7, New York.